23 December 1977, Volume 198, Number 4323 SCIENCE

Oxygen and Hydrogen Isotopic Ratios in Plant Cellulose

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Summary. The variations of the D/H and $^{18}O/^{16}O$ ratios of nonexchangeable hydrogen and oxygen in plant cellulose reveal systematic differences between aquatic and terrestrial plant groups. The slope of δD versus $\delta^{18}O$ of cellulose from a variety of aquatic plants is close to 8 (the meteoric water value), while the slope for a number of terrestrial species is greater than or equal to about 24. Two models involving incorporation of CO_2 and H_2O into cellulose precursors are proposed to account for these differences. Effects of evaporative transpiration on the isotopic composition of water in leaves are measured and discussed in the context of these models.

The deuterium/hydrogen ratio of the hydrogen bound to carbon (C-H hydrogen) in cellulose of both terrestrial and aquatic plants reflects the D/H ratio of the water used by the plant in the synthesis of its cellulose (*1*). Because the D/H ratio of meteoric water at any location is directly related to the climatic temperature, the D/H ratio of the C-H hydrogen in cellulose of plants is also related to climatic temperature. Climatic temperature variations have been estimated from D/H ratios of cellulose in tree rings from a bristlecone pine and from a Scotch pine (*2*) and cellulose from wood samples which grew during the Wisconsin glacial period (*3*).

It is also well known that the $\delta^{18}O$ and the δD (*4*) in meteoric waters are related by the simple equation (*5*)

$$\delta D = 8\delta^{18}O + 10 \quad (1)$$

Consequently it might be anticipated that the $^{18}O/^{16}O$ ratio of plant cellulose would also reflect climatic temperature. This is expected for aquatic plants, for which the only possible sources of oxygen for cellulose synthesis are the water and CO_2 dissolved in the aquatic medium. The dissolved CO_2 is almost certain to be in isotopic equilibrium with the aquatic medium because equilibration can take place in a matter of hours (*6*). Thus the $^{18}O/^{16}O$ ratio of the oxygen of the dissolved CO_2 varies directly as the $^{18}O/^{16}O$ ratio of the waters. It follows that the $^{18}O/^{16}O$ ratio of the cellulose of aquatic plants should have an approximately one-to-one correlation with the $^{18}O/^{16}O$ ratio of the water, regardless of the oxygen isotopic fractionation that might be associated with the synthesis of the cellulose.

Nonaquatic plants obtain their CO_2 from the atmosphere, and the $\delta^{18}O$ of atmospheric CO_2 is not related to the $\delta^{18}O$ of meteoric water. Atmospheric CO_2 has a $\delta^{18}O$ value of about 41 per mil and is nearly uniform isotopically throughout the world (*7*). Thus, the $\delta^{18}O$ of that part of the cellulose oxygen originating from the atmospheric CO_2 would not be sensitive to climate unless the CO_2 isotopically equilibrates with the water in the plant cells or there is a temperature-sensitive oxygen isotopic fractionation associated with the fixation of atmospheric CO_2. If some of the cellulose oxygen is derived from the environmental water, the $\delta^{18}O$ of the cellulose in terrestrial plants would be sensitive to climate, but to a lesser degree than the cellulose in aquatic plants.

Another factor affecting the $^{18}O/^{16}O$ and D/H ratios of cellulose in terrestrial plants is the evaporative transpiration of water through their leaves. The evaporative transpiration increases both the δD and the $\delta^{18}O$ of the plant cell water relative to local meteoric water (*8, 9*). The water used in cellulose synthesis may thus have more positive $\delta^{18}O$ and δD values than local meteoric water.

In this article we describe several experiments done to ascertain the relationships between climate and the $\delta^{18}O$ values of cellulose in aquatic and terrestrial plants and between the $\delta^{18}O$ values of cellulose and the $\delta^{18}O$ values of water and atmospheric CO_2 used by the plants. These experiments involved analyses of the δD and $\delta^{18}O$ of water vacuum-distilled from freshly cut leaves from plants, and of cellulose extracted from both terrestrial and aquatic plants from a variety of locations. In addition to the usefulness of this isotopic approach in obtaining paleoclimatic information, some insight was obtained into the sources of oxygen used by different plants for synthesis of cellulose.

Experimental Procedure

1) All the available plant waters were vacuum-distilled from freshly cut leaves or pine needles. The leaves or needles were cut from a tree and immediately inserted into glass vessels and sealed from the atmosphere. This procedure ensured the extraction of plant water contaminated with a negligible quantity of atmospheric water vapor. The δD and $\delta^{18}O$ values of the water were determined by well-established methods (*10, 11*).

2) Deuterium/hydrogen ratios of C-H hydrogen in the cellulose were analyzed by a method described previously (*1*). In this method pure cellulose nitrate, which contains only the C-H hydrogen of cellu-

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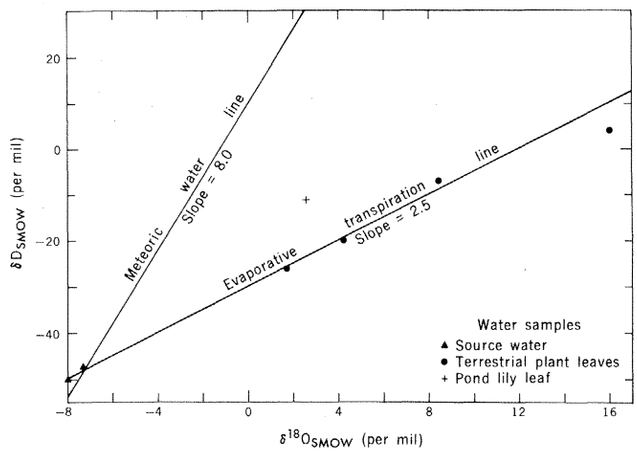

Fig. 1. Relationship between the δD and $\delta^{18}O$ of water extracted from leaves of terrestrial plants and from leaves of a pond lily compared to the δD-$\delta^{18}O$ relationship for meteoric waters (*5*). The source water was the water used for irrigation.

lose, is extracted from nitrated wood. In turn, the cellulose nitrate is combusted and the water formed reduced to give H_2 gas for mass spectrometric analysis.

3) For the $\delta^{18}O$ analyses the cellulose was extracted from the plants by a delignification procedure, using NaClO and NaOH solutions (*12*). To ensure the purity of the final cellulose, the lipids and acetone-soluble components were extracted prior to the delignification procedure.

The oxygen of the cellulose was extracted as CO_2 for the mass spectrometric analysis by two different methods. One method involved heating about 15 milligrams of cellulose in a closed evacuated nickel vessel at 1100°C. At this temperature any H_2 gas formed in the reaction diffuses from the vessel through its heated walls out into the atmosphere. The complete removal of hydrogen prevents any hydrogen compounds from forming in the nickel reaction vessel and results in the cellulose oxygen being totally converted to CO and CO_2. The CO is isolated and converted quantitatively to CO_2 by a well-established electric discharge method (*13*), combined with the original CO_2, and analyzed mass spectrometrically.

Some of the cellulose oxygen was extracted by reacting the cellulose with a threefold excess of $HgCl_2$ at 400°C according to a modified version of the method of Rittenberg and Ponticorvo (*14*). This modified procedure, when applied to pure cellulose, gives CO_2 and CO whose combined oxygens give $\delta^{18}O$ values within ±0.3 per mil of the values for the oxygen extracted by the nickel pyrolysis method. Some isotopic exchange between CO_2 and Pyrex walls of the reaction vessel was observed at 525°C but did not occur when the reaction was run at 400°C. About 5 to 20 percent of the cellulose oxygen is not recovered as CO or CO_2 and is probably in the form of H_2O, but the loss of this oxygen does not appear to affect the reproducibility of the $\delta^{18}O$ analysis or the agreement of the $\delta^{18}O$ values with those attained by pyrolysis in the nickel reaction vessel. This procedure will be reported in detail elsewhere.

The δD and $\delta^{18}O$ values are reported relative to SMOW (*4*). The $\delta^{18}O$ analyses of the cellulose are reproducible within ±0.3 per mil and the δD analyses within ±2 per mil.

Results and Discussion

The δD and $\delta^{18}O$ of water in plants. Figure 1 shows the relationship between the δD and $\delta^{18}O$ of the water vacuum-distilled from various plant leaves collected at the Los Angeles State and County Arboretum, Arcadia, California. Also included are two water samples from a pond and a tap used to water the plants. The samples were collected on a warm dry afternoon in July 1975. As Fig. 1 shows, the data for the two source water samples lie close to the meteoric water line. The data points for the leaf waters from terrestrial plants do not fall on the meteoric water line but on a curve whose slope is approximately 2.5. The latter curve intersects the meteoric water line at the values of the δD and $\delta^{18}O$ of the water which is used by the plants. This curve through the leaf water data reflects the effect of nonequilibrium evaporation (*8, 9*) on the δD and $\delta^{18}O$ of water in the plant accompanying evaporative transpiration through the leaves of the plant. Bricout (*9*) obtained a slope of about 4 for the relationship between the δD and $\delta^{18}O$ of water in oranges. It is possible that partial equilibration between atmospheric water and the fruit juice analyzed by Bricout took place, counteracting some of the kinetic isotope effects of evaporative transpiration apparent in the δD and $\delta^{18}O$ of water distilled from plant leaves.

A line joining the isotopic composition of the water from the leaves of the pond lily to that of the pond water has a slope steeper than 2.5. The pond lily grows partially on the water surface and partially immersed, and thus grows in a more humid environment than the terrestrial plants. The slopes of lines through the δD and $\delta^{18}O$ values of leaf water might be expected to be steeper in regions of greater relative humidity because kinetic isotope effects are reduced when water evaporates at higher relative humidity.

The isotopic compositions of plant leaf waters in Fig. 1 reflect conditions during the specific day in which the leaf samples were collected and thus probably are not average isotopic compositions of water used by the plants during synthesis of the cellulose analyzed. However, it seems reasonable to suppose that on the average the waters in the plant cells during photosynthesis also have δD and $\delta^{18}O$ values which lie off the normal meteoric water line. For convenience, the value of 2.5 will be used in subsequent considerations of the effect of evaporative transpiration on the δD and $\delta^{18}O$ of plant leaf water, in spite of the fact that the value for this slope is probably variable and is affected by such factors as relative humidity, wind velocity, and air temperature (*8*). These are all climatic factors of great interest that may possibly be inferred by a combined analysis of the $\delta^{18}O$ and δD of cellulose in plants. To determine how readily such information can be obtained it is probably necessary to analyze a variety of plants grown in controlled environments. However, isotope analyses of some naturally occurring samples discussed below provide encouraging indications that such information may indeed be obtained from the combined δD and $\delta^{18}O$ data for cellulose from terrestrial plants.

Relationship between the $\delta^{18}O$ of cellulose oxygen from aquatic plants and the $\delta^{18}O$ or δD of their aquatic media. The δD values of the C-H hydrogen and the $\delta^{18}O$ values of total oxygen in cellulose from seven aquatic plants as well as the measured and the inferred δD values of the water in which the plants grew are given in Table 1. The inferred δD of the water was obtained from the measured δD of the C-H hydrogen in the cellulose by using the equation (*1*)

$$\delta D_{(water)} = \delta D_{(C-H)} + 22 \qquad (2)$$

This equation represents the relationship between the δD values of C-H hydrogen in cellulose from more than 20 different species of terrestrial and aquatic plants

and the δD of the environmental water used by the plants. The locations of these plants ranged from the subarctic Lake Kluane area in Yukon Territories to the semitropical Miami Beach area. A least-squares calculation on these data shows that $\delta D_{(H_2O)}$ calculated from this equation has an uncertainty of ±11 per mil (1 standard deviation). This large uncertainty is probably due to the fact that evaporative transpiration affects the leaf water δD to different extents in different climates (see below) and to possible temperature effects on the isotopic fractionation involved in the fixation of hydrogen in the synthesis of cellulose by plants.

For marine plants it is possible to determine the relationship between the $\delta^{18}O$ of the cellulose and the $\delta^{18}O$ or the δD of the water in which the plants grew because the $\delta^{18}O$ and δD values of normal marine water are nearly constant throughout the year and are known accurately. Some of the freshwater aquatic plants grew in lakes whose isotopic compositions were not constant throughout the growth period of the plant. Consequently, single samples of some lake waters were not sufficient for assigning $\delta^{18}O$ values for the aquatic media. This was particularly the case for the dammed lakes in the Sierra Nevada from which three plant samples listed in Table 1 were collected. Consequently the $\delta^{18}O$ or the δD of each freshwater lake was inferred from the δD of the C-H hydrogen in the corresponding cellulose by using Eqs. 1 and 2, and as pointed out above the inferred δD and $\delta^{18}O$ values of the aquatic media would have uncertainties of ±11 and ±1.4 per mil, respectively.

The relationship between the δD of the aquatic media and the $\delta^{18}O$ of the cellulose of all the aquatic plants in Fig. 2 falls on or close to a straight line (curve 1) which is drawn parallel to the meteoric water line. The aquatic plant from Bridgeport Lake, *Polygonum natans*, has parts which float on the water. It is possible that, as was the case for the isotope data for the plant water of the water lily (Fig. 1), evaporative transpiration in *P. natans* may have caused the displacement of the $\delta^{18}O$ from curve 1 toward a higher value (see datum point on curve 2). The δD and $\delta^{18}O$ cellulose of aquatic plants growing completely submerged cannot be modified by evaporative transpiration unless the body of water in which the plants grew has itself suffered nonequilibrium evaporation (as occurs during periods where evaporation substantially exceeds precipitation).

Data in Table 1 allow an estimation of the oxygen isotope fractionation factor between the cellulose in aquatic plants and the water in which it was synthesized. The $\delta^{18}O$ values of the cellulose from turtle grass, *Phyllospadix*, and Eurasian water milfoil are, respectively, 28.2, 27.6, and 20.1 per mil. The waters in which they grew are probably constant to within 1 per mil throughout the growing season, with $\delta^{18}O$ values of 1, −0.4, and −7.6 per mil, respectively. The fractionation factors

$$\alpha = \frac{1 + \delta^{18}O_{cellulose}/1000}{1 + \delta^{18}O_{H_2O}/1000}$$

are thus 1.027, 1.028, and 1.028—excellent agreement for three different spe-

Table 1. Isotopic compositions of the nonexchangeable hydrogen and oxygen in cellulose from aquatic and terrestrial plants; also included are the measured and inferred isotopic compositions of the environmental waters associated with the plants.

Sample (type)	Location (δD of nearby water, per mil)	δD (per mil)		$\delta^{18}O$ (per mil)
		Cellulose nitrate	Water, inferred (measured)	Cellulose
Turtle grass (aquatic)	Open ocean off Puerto Rico (+7)	−3	(+7)	28.3*
Turtle grass (aquatic)	Open ocean near Miami, Florida (+7)	−1	(+7)	28.1
Phyllospadix (aquatic)	Open ocean near Santa Catalina, California (−3)	−40	(−3)	27.6
Eurasian water milfoil (aquatic)	Lake Mendota, Wisconsin (−51)	−71	−49	20.1*
Elodia canadensis (aquatic)	Twin Lake, California (−110)	−80	−58	17.6
Aquatic plant (meadow foam ?)	Grant Lake, California	−95	−73	14.2*
				14.2
Polygonum natans (aquatic)	Bridgeport Lake, California (−107)	−110	−88	17.3
Red mangrove	Island off Puerto Rico	+18	+40	33.0
Red mangrove	Open ocean near Miami, Florida (+7)	−7	+15	29.2*
Culture-tube cotton	Commercial	−37	−15	28.2*
Oak tree	Houston, Texas (−24)	−43	−21	30.5
Cotton linter	Commercial	−46	−24	28.3*
Pinus sylvestris	Loch Affric, Scotland (−42)	−54	−32	29.2
Whatman cellulose	Commercial	−72	−50	27.5*
Hard maple	Near Spring Green, Wisconsin River (−69)	−84	−62	26.6
Grass (unidentified)	Near Spring Green, Wisconsin River (−69)	−85	−63	27.7
Monterey pine	Asilomar, California	−85	−63	30.2*
				30.2
Jeffrey pine	Mount Whitney	−86	−64	30.8
White birch	Marsh near Oconto, Wisconsin (−65)	−92	−70	24.4
Sigma α-cellulose	Commercial	−94	−72	27.6*
				26.5
Bristlecone pine	White Mountains, California	−95	−73	33.1
Grass (unidentified)	Marsh near Oconto, Wisconsin (−65)	−103	−81	25.8
Coulter pine	Kalamalka Lake, British Columbia (−97)	−103	−81	27.8
Pine tree	Seeley Lake, British Columbia (−136)	−122	−100	22.8
				23.0*
Sedge	Nymph Lake, Colorado (−104)	−140	−118	22.9
White spruce	Lac des Roches, British Columbia (−113)	−133	−111	23.3
Lombardy poplar	Near Okanogan Lake, British Columbia (−106)	−142	−120	24.2
Quaking aspen	Stuart Lake, British Columbia, southeast corner (−144)	−154	−132	20.0
Lodgepole pine	Seeley Lake, British Columbia (−136)	−154	−132	21.5
White spruce	Kluane Lake, Yukon Territory (−174)	−181	−159	20.6
				20.2*

*Samples were prepared by the nickel pyrolysis method.

cies of aquatic plants. The isotope data for aquatic plants which fall on curve 1 in Fig. 2 also give fractionation factors for cellulose fixation of about 1.027.

These data do not permit a detailed understanding of the factors which determine the oxygen isotope fractionation factor associated with the cellulose synthesis. However, a possible interpretation suggested by the value of 1.027 is that one-third of the oxygen in the cellulose of aquatic plants comes from the environmental water, that two-thirds comes from the isotopically equilibrated dissolved CO_2, whose $\delta^{18}O$ is 42 per mil (*15*) relative to that of the water at 25°C, and that oxygen from each of these two sources is incorporated into the cellulose without any isotopic fractionation. The chemical basis for this model (henceforth referred to as model A) is that the reaction which is responsible for CO_2 fixation in most plants involves the addition of one molecule of CO_2 and one molecule of H_2O (*16*). The relationship observed between the $\delta^{18}O$ of the water and that of the cellulose in terrestrial plants does not at this time seem to contradict such an explanation (see below).

Two of the data points for the aquatic plants lie significantly off curve 1 in Fig. 2. Assuming that model A is applicable, these deviations could be due to at least two factors. The $\delta^{18}O$ of the dissolved CO_2 varies with temperature (*15*), and the δD of the C-H hydrogen could also vary significantly from species to species depending on the effect of temperature or the isotopic fractionation associated with hydrogen fixation into cellulose. The possibility that nonequilibrium evaporation occurred in the lakes in which these plants grew cannot be ruled out.

The effect of temperature on the fractionation factor for the dissolved CO_2-H_2O system is quite large, 0.25 per mil per degrees Celsius (*15*). Hence the $\delta^{18}O$ of the dissolved CO_2 available for cellulose synthesis would differ by 3 per mil just because of a temperature difference of 12°C. Thus, if model A is correct, cellulose from two plants grown in water of the same $\delta^{18}O$ but at temperatures different by 12°C would have $\delta^{18}O$ values differing by 2 per mil (that is, two-thirds of 3 per mil). This temperature effect is illustrated by the difference between curve 1 and curve 2 in Fig. 2.

Relationship between the $\delta^{18}O$ of the oxygen in the cellulose of terrestrial plants and the inferred δD and $\delta^{18}O$ of the environmental H_2O. Table 1 also contains all the data for the terrestrial plants. The $\delta^{18}O$ values of the cellulose of terrestrial plants are plotted in Fig. 2 against the inferred δD values of meteoric H_2O used by the plants. Whereas in aquatic plants (curve 1) the variation of δD in cellulose is approximately eight times as large as the variation in $\delta^{18}O$, in terrestrial plants this ratio is variable but is closer to 20. The $\delta^{18}O$ of the terrestrial plant cellulose can be accounted for by assuming that the cellulose of terrestrial plants was formed by the same process as the cellulose of aquatic plants, except that in the case of terrestrial plants two-thirds of the oxygen originated from atmospheric CO_2 [$\delta^{18}O$ approximately 41 per mil (*7*)] rather than dissolved CO_2. The other one-third of the oxygen originated, as before, from the water of the plant. This is referred to as model B. As shown above, the $\delta^{18}O$ of the water used by the plants can be estimated with an accuracy of about ± 1.4 per mil from the δD values of meteoric water, inferred, in turn, from the δD of the C-H hydrogen in the cellulose.

Thus

$$\delta^{18}O_{cellulose} = \tfrac{2}{3}(41) + \tfrac{1}{3}\delta^{18}O_{water}$$

and rearranging and combining with Eq. 1.

$$\delta D_{(water)} = 24\,[\delta^{18}O_{(cellulose)} - 27.3] + 10 \qquad (3)$$

Equation 3 is plotted as curve 3 in Fig. 2. The curve passes near most of the data points for terrestrial plants. Most points which lie off curve 3 deviate toward more positive $\delta^{18}O$ values. This deviation from the calculated curve is exactly the effect that evaporative transpiration would cause.

The $\delta^{18}O$-δD curves for the cellulose in aquatic plants (curve 1) and in terrestrial plants (curve 3) meet at the point where the δD and $\delta^{18}O$ values of the water are equal to zero and the $\delta^{18}O$ value of the cellulose is equal to 27.3 per mil. At this point the $\delta^{18}O$ of a atmospheric CO_2 and the $\delta^{18}O$ of dissolved CO_2 are about the same, approximately 41 per mil.

Striking features of the data in Fig. 2 for the terrestrial plants are the scatter in the data points and the low sensitivity of the $\delta^{18}O$ values of cellulose to variation of either the δD or the $\delta^{18}O$ of the source water. Consequently, the effect of climatic temperature on the $\delta^{18}O$ of cellulose oxygen is markedly lower in terrestrial than in aquatic plants. For example, the δD of C-H hydrogen in cellulose of a subarctic Yukon spruce is 178 per mil lower than the δD of a tropical Miami terrestrial plant, a red mangrove. On the other hand, the $\delta^{18}O$ of the Yukon spruce cellulose is only 9 per mil lower than that of the Miami red mangrove. The ratio of the variation in δD to the variation in

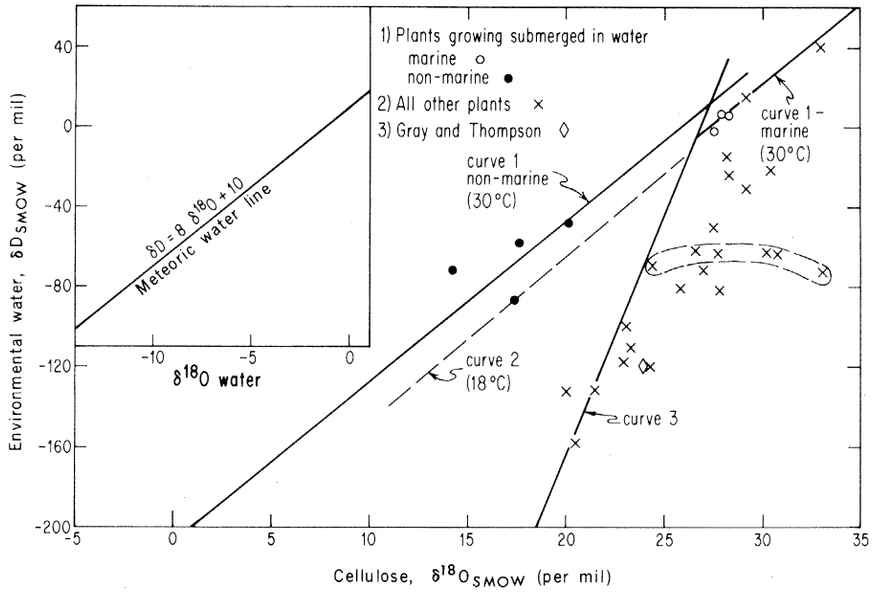

Fig. 2. Relationships between the $\delta^{18}O$ of the cellulose (from both aquatic and terrestrial plants) and the δD of the meteoric water used by the plants. The δD of the meteoric water was either measured directly or inferred from the δD of the C-H hydrogen in the cellulose by using Eq. 2. The relationship between the $\delta^{18}O$ values of the cellulose and the $\delta^{18}O$ of the water used by the plant can be calculated from these graphs by noting that the $\delta^{18}O$ of the water can be calculated from the δD of meteoric water by using Eq. 1. Note that curve 1 for the marine samples is displaced downward by a constant 10 per mil as compared to curve 1 for the nonmarine samples. This displacement occurs because for average ocean water $\delta D = 0$ and $\delta^{18}O = 0$, but for meteoric waters, when $\delta^{18}O = 0$, $\delta D = 10$ per mil. The continuation of curve 1 to more positive values is based on the assumption that further evaporation of ocean waters under very humid conditions increases its δD eight times as much as its $\delta^{18}O$.

$\delta^{18}O$ for the two samples is approximately 20 instead of the value of 8 observed for meteoric waters. Since there is only a 9 per mil spread in $\delta^{18}O$ values of terrestrial cellulose between Miami and Yukon, evaporative transpiration can be very important in affecting the $\delta^{18}O$ record of terrestrial plant cellulose. Possibly, the major use of the $\delta^{18}O$ of terrestrial plant cellulose in climatic studies may be as an indicator of evaporative transpiration, which itself is a function of relative humidity and the amount of rainfall in the growth area rather than of temperature.

The effect of humidity on $\delta^{18}O$ is illustrated qualitatively by the data enclosed in the dashed curve in Fig. 2. The δD values of all these samples are similar. The least positive $\delta^{18}O$ value, 24.4 per mil, is for cellulose from a white birch tree which grew in a humid marsh area near Oconto, Wisconsin. The next five samples, in order of increasing $\delta^{18}O$ values, grew in regions in which relative humidity during the spring and summer growing season correspondingly decreased. They are a hard maple from Spring Green, Wisconsin (26.6); a grass from the same area (27.7); a Monterey pine from a coastal area near Asilomar, California (30.2); a pine from an elevation of about 2600 meters on Mount Whitney (30.8); and a bristlecone pine from about 3300 meters on White Mountain (33.1). This series of samples illustrates, at least qualitatively, that humidity and the amount of precipitation in the growth areas may affect the relationship between the $\delta^{18}O$ and δD of the cellulose in plants. Undoubtedly, there are other factors such as the species of the plant which will affect the relationship between $\delta^{18}O$ and δD.

The possibility that the CO_2 in terrestrial plants is partially equilibrated with plant cell water prior to its fixation cannot be ruled out by our data. If complete equilibration occurred, then in the absence of evaporative transpiration the isotopic data for terrestrial plants would show relationships between δD and $\delta^{18}O$ predicted by model A, which was initially derived for aquatic plants. Data points would plot in the region of curves 1 and 2 in Fig. 2. The deviation of δD-$\delta^{18}O$ data points from that region could be entirely due to the effect of evaporative transpiration and model B would not apply. Figure 1 shows that very large ^{18}O enrichments in the plant water (~ up to 24 per mil) can be caused by the evaporative transpiration process, and thus in principle the data points for terrestrial plants could reflect formation under conditions of model A, with major shifts in the composition of the plant water caused by evaporative transpiration.

Partial equilibration between CO_2 and plant cell water in terrestrial plants would result in cellulose with an isotopic composition that would plot in the area between curves 1 and 3 in Fig. 2. The absence of data lying in that region suggests that CO_2 may be fixed very quickly on diffusion into the plant leaf. However, there is no information as to how rapidly the $\delta^{18}O$ of atmospheric CO_2 is exchanged on absorption of the CO_2 into the plant.

The relative merits of model A (complete equilibration of CO_2 with plant water) and model B (no equilibration of CO_2 with plant water) in explaining the $\delta^{18}O$ of terrestrial plants can be compared by determining which of these two models provides the best estimate of the $\delta^{18}O$ and δD of the meteoric waters in which the plants grew from the δD and $\delta^{18}O$ values of the cellulose. The observed values of δD and $\delta^{18}O$ for cellulose can be corrected for the isotopic changes in the plant cell water due to evaporative transpiration effects, assuming for the purpose of discussion a slope of 2.5 for δD and $\delta^{18}O$ changes due to evaporative transpiration. Knowledge of the oxygen and hydrogen fractionation factors between cellulose and water then permits the estimation of the δD and $\delta^{18}O$ of the meteoric water used by the plant. Estimates of the δD and $\delta^{18}O$ of meteoric water from the δ values of the cellulose were made with model A and model B. The model which gave the isotope values closest to the actual δ values of the environmental water is presumably the correct model. Figure 3 illustrates how these estimates can be made.

For instance, consider the δD of the inferred cell water and the $\delta^{18}O$ value of the bristlecone cellulose, which are -73 and 33.1, respectively. According to model A the high δD and $\delta^{18}O$ values for the cellulose are due entirely to evaporative transpiration effects. The data point for the bristlecone pine can be extrapolated back to curve 1 in Fig. 3 by drawing a connecting line of slope 2.5. The resulting δD value for the meteoric water would have been -140 per mil and $\delta^{18}O$ for the cellulose 8.5 per mil if evaporative transpiration had not occurred. Based on the proposed oxygen isotope difference of 27 per mil between cellulose and water, the $\delta^{18}O$ of the meteoric water would be -18.5 per mil.

If model B is used, the increase in the $\delta^{18}O$ of the cell water due to evaporative transpiration is three times as large as the increase in the $\delta^{18}O$ of the cellulose, since according to this model one-third of the cellulose oxygen originates from the water and two-thirds of it originates from atmospheric CO_2, whose $\delta^{18}O$ value is 41 per mil. The evaporative transpi-

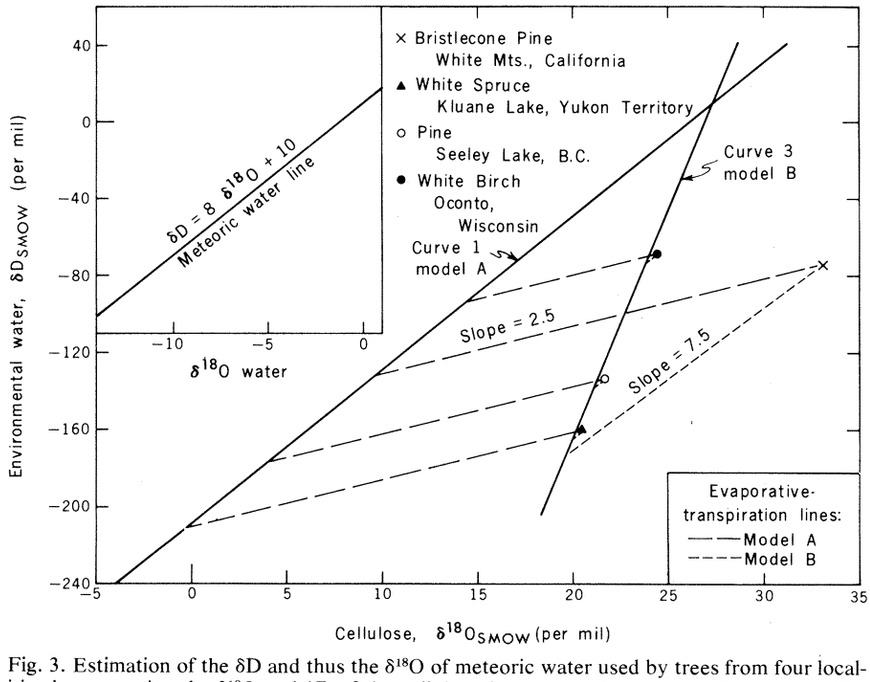

Fig. 3. Estimation of the δD and thus the $\delta^{18}O$ of meteoric water used by trees from four localities by correcting the $\delta^{18}O$ and δD of the cellulose for evaporative transpiration effects. The slopes of the evaporative transpiration lines based on model A are assumed to be 2.5, although the value should probably be lower for the arid White Mountains area and higher for the other three more humid areas. In these three cases the δD of the meteoric waters estimated from model B are much closer to the actual measured values for nearby bodies of water (Table 1) than are the values estimated from model A.

ration effects on water correspond to a slope of 7.5 when plotted on a graph of δD in water against $\delta^{18}O$ in cellulose. Thus the data point for the bristlecone pine in Fig. 3 is joined to curve 3 by a line of slope 7.5. The intersection on curve 3 shows that the δD of the plant water would have been about -174 per mil and the $\delta^{18}O$ of the cellulose oxygen would have been about 19.6 per mil if no evaporative transpiration had taken place. This means that the $\delta^{18}O$ of the meteoric water used by the plant, as calculated from Eq. 1, would be approximately -23 per mil.

The δ values for meteoric water used by the bristlecone pine as estimated by both models appear rather low but not entirely out of the range of the possible δ values for the precipitation of the area. It is also possible that the variation in the $\delta D/\delta^{18}O$ ratio due to evaporative transpiration is closer to or less than 2 in this area of very low humidity in the White Mountains. The estimated δD and $\delta^{18}O$ values for the meteoric water are less negative if a slope of less than 2.5 is used. In both cases, the models give similar results for the estimated δ values of meteoric water in the neighborhood of the bristlecone locality, and the bristlecone cellulose δD and $\delta^{18}O$ data cannot be used to determine which model is valid for terrestrial plants.

The data points of Fig. 3 which fall close to curve 3 are more definitive in suggesting that the mechanism described by model B gives the more correct δ values for meteoric water in the locality of these samples. For example, the cellulose from the Yukon spruce tree near Lake Kluane gives a δD value for meteoric water of -160 per mil according to model B but gives a value of -240 per mil according to model A. The measured δD value for water in Lake Kluane is -174 per mil. Considering the uncertainties and the assumptions that have been made, the δD values calculated with model B are in good agreement with the measured ones. Similar support for model B can be obtained by using the data for samples from Wisconsin and for most of the samples from British Columbia, whose δD and $\delta^{18}O$ data points are close to curve 3.

In all these cases it has been assumed that the ratio of the change in δD to the change in $\delta^{18}O$ due to evaporative transpiration is similar to that observed in Arcadia, California, which has a semiarid climate. Since the climates in British Columbia, the Yukon, and Wisconsin are more humid, it is highly probable that the slopes for the samples from these localities are much higher than the value of 2.5 assigned for the calculations. If so, the discrepancy between the measured δD and the δD of the lake water calculated with model A would be even greater than that stated above, and thus model A appears to be even less acceptable (*17*).

The δD and $\delta^{18}O$ of cellulose from terrestrial plants can give a good qualitative estimate of the amount of evaporative transpiration in an area. Thus, the isotopic compositions of terrestrial plants in an arid region may be expected to plot farther to the right of curve 3 than those of plants from more humid regions. However, inasmuch as some plants such as the succulents may have adapted to their environments by minimizing evaporative transpiration, the $\delta^{18}O$ and δD relationship of the cellulose in these plants may differ from that in other plants. Indeed, one of the more interesting applications of the oxygen and hydrogen isotope analyses of cellulose in plants may be to study how efficiently plants use the water available to them and how much of the water is lost by evaporative transpiration. The effects of species of plants and of other conditions which control the final δD and $\delta^{18}O$ values of the cellulose synthesized are subject to experimental investigations in the laboratory.

Two other laboratories have published $\delta^{18}O$ data on wood or cellulose. Libby *et al.* (*18*) analyzed total wood from tree rings. The δD and $\delta^{18}O$ values of such samples are markedly affected by the relative proportions of the many different compounds present in wood. It is doubtful whether their data have any relevance to climate. Some additional comments on their data are found elsewhere (*19*).

The paper of Gray and Thompson (*20*) gives $\delta^{18}O$ values of cellulose, as determined by the nickel reaction vessel method. Their samples are from successive rings in a spruce tree which grew in Edmonton, Alberta, Canada. They obtain a linear relationship between $\delta^{18}O$ and the average yearly temperature in Edmonton. We do not understand the basis for this relationship. Analysis of the δD of their samples would prove useful, since as we have shown here the $\delta^{18}O$ is affected by evaporative transpiration and humidity as well as the $\delta^{18}O$ of the meteoric water.

The average value of $\delta^{18}O$ for their tree is 24 per mil. The δD for average meteoric water in Edmonton is estimated from published data to be about -120 per mil (*21*). The point shown as a diamond in our Fig. 2 represents their datum quite well and lies among the data for other terrestrial plants grown in northern climates. Contrary to what Gray and Thompson assume, the cellulose or its precursors need not have been formed during the winter, nor would the CO_2 have had to be in equilibrium with meteoric water prior to its fixation to explain their observations.

Conclusion

Analyses of δD and $\delta^{18}O$ in water extracted from leaves and pine needles of plants grown in a semiarid region of California show a linear relationship with a slope of 2.5, which is lower than the slope of 8 observed for meteoric water collected throughout the world. The lower slope results from kinetic isotope effects during evaporative transpiration of plant water.

Similarly, δD and $\delta^{18}O$ were analyzed in unexchangeable hydrogen and oxygen in cellulose from aquatic and terrestrial plants and compared to the δD and $\delta^{18}O$ values for the water used by the plants. In the case of aquatic plants, the isotope fractionation factor between the oxygen in the cellulose and that in the water medium is 1.027 to 1.028. A model which accounts for this fractionation factor is that two-thirds of the cellulose oxygen comes from the dissolved CO_2, whose $\delta^{18}O$ value is 42 per mil (at 25°C) relative to the water, and that one-third originates from the oxygen of the water. The temperature coefficient for this fractionation factor should be 0.16 per mil per degrees Celsius. This value is two-thirds of 0.25 per mil per degrees Celsius, which is the temperature coefficient for the fractionation factor of the CO_2-H_2O system.

Both δD and $\delta^{18}O$ of the cellulose in aquatic plants are directly related to the isotopic composition of the water medium. This relationship could be affected by temperature.

The relationship between $\delta^{18}O$ and δD in cellulose from terrestrial plants is complicated by the effect of evaporative transpiration on the isotopic composition of plant water as well as by the possibility that there were various degrees of isotopic equilibration of atmospheric CO_2 with the plant water prior to the CO_2 fixation into cellulose. In each case for which a comparison can be made, when an aquatic and a terrestrial plant have similar δD values, the $\delta^{18}O$ values of the terrestrial plants are higher by 4 to 16 per mil. This enrichment is very large when compared to the total $\delta^{18}O$ range of 13 per mil observed for terrestrial plants grown in such varied climates as those of the Yukon Territories, Puerto Rico, and

White Mountain, California. Therefore the $\delta^{18}O$ of the cellulose does not serve as a sensitive or reliable indicator of temperature between regions or species.

Two models have been proposed to account for the $\delta^{18}O$ of the cellulose from terrestrial plants. In model A it is simply assumed that the difference between the $\delta^{18}O$-δD relationship of aquatic plants and that of terrestrial plants is due to the effect of evaporative transpiration on $\delta^{18}O$ and δD of the plant leaf water in terrestrial plants. In model B it is assumed that some of the $\delta^{18}O$ increase in the terrestrial cellulose is due to the use by terrestrial plants of atmospheric CO_2, whose $\delta^{18}O$ of 41 per mil is not altered isotopically during its fixation. Model B seems to be more compatible with the data. Nevertheless, these are tentative models which must be analyzed more rigorously by laboratory experiments.

The δD of cellulose by itself most closely reflects the isotopic composition of meteoric waters and thus climatic temperature. Together, δD and $\delta^{18}O$ can be used to evaluate the effect of the humidity of the growth area during the time when the plant grew. The oxygen and hydrogen isotope data for cellulose and other components of plants are potentially powerful tools for evaluating the relationship between plant growth and the use of water by the plants.

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$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where for $\delta^{18}O$, $R = {}^{18}O/{}^{16}O$; and for δD, R = D/H. The standard is standard mean ocean water (SMOW).
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16. The reaction for the CO_2 fixation in C_3-type plants is

ribulose 1,5-diphosphate + CO_2 + H_2O → 2 (3-phosphoglycerate)
17. It has been assumed that virtually no oxygen isotopic fractionation occurs during the conversion of CO_2 and H_2O into cellulose. This assumption may not be correct. If oxygen isotopic fractionation does occur, then for model A

$$\delta^{18}O_{cellulose} = 2/3\,[(\delta^{18}O_{H_2O} + 41) + a] + 1/3\,[\delta^{18}O_{H_2O} + b]$$

where $\delta^{18}O_{H_2O} + 41 = \delta^{18}O_{CO_2aq}$. For model B

$$\delta^{18}O_{cellulose} = 2/3[41 + a] + 1/3[\delta^{18}O_{H_2O} + b]$$

where a is the $\delta^{18}O$ change due to fractionation during CO_2 incorporation and b is the $\delta^{18}O$ change due to fractionation during H_2O incorporation. Models A and B could be considered special cases where $b = -2a$; that is, incorporation of H_2O into the cellulose involves an oxygen isotope fractionation twice as large as and in the opposite direction to the oxygen isotopic fractionation associated with CO_2 incorporation into cellulose.
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22. We wish to thank R. Potter, C. Kendall, M. Johnson, and J. Way for making many of the analyses. M. DeNiro helped collect samples, provided scientific advice, helped in many of the operations carried out, and critically read the manuscript. H.-W. Yeh provided both technical and scientific help throughout the project. Discussion with J. F. Bonner was most welcome throughout this research. Samples were provided by C. Emiliani of the University of Miami, H. D. Bruhn of the University of Wisconsin, and H. P. Taylor of the California Institute of Technology. The work was supported by the National Science Foundation, Office of Climate Dynamics, grant number ATM76-03972. This is contribution 2897 from the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena 91125.

Metals as Regulators of Heme Metabolism

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Heme is a red pigment comprised of four subunits called pyrroles; these subunits are joined together as a single large tetrapyrrole (porphyrin) ring structure. At the center of this porphyrin a metal atom is chelated. In higher organisms the chelated metal is usually iron and the porphyrin is protoporphyrin IX; however, in more primitive species other metalloporphyrin complexes are also formed, for example copper-uroporphyrin is found in the feathers of the Turaco bird (*1*) and cobalt-coproporphyrin is formed in *Propionibacterium arabinosum* (*2*). In order that the metalloporphyrin be of metabolic significance, the central metal ion must be a transition element and capable of undergoing reversible changes in oxidation state (for example, $Fe^{2+} \rightleftarrows Fe^{3+}$, $Cu^{1+} \rightleftarrows Cu^{2+}$, $Co^{2+} \rightleftarrows Co^{3+}$).

In physiological systems heme is bound to certain proteins, and these heme proteins bind oxygen at the site of the metal atom or they function as components of membrane-bound electron transport chains. The ability of heme proteins to carry out these functions is a property of the chelated central metal ion. The porphyrin ring enhances the catalytic activity of the metal, and this activity is further augmented by the complexing of the porphyrin with its apoprotein moiety. The extent of enhancement of the catalytic properties of metals in heme-protein complexes may be of the order of 10^5 to 10^{10}, as compared with unchelated elements (*3*). The porphyrin ring may also serve as an inter- and intracellular carrier of metal ions to regulatory sites for the rate-limiting enzymes of heme synthesis and heme degradation. Porphyrins without a central metal iron are incapable of carrying out those metabolic functions attributed to heme; however, various forms of free porphyrins may have other biological purposes, such as serving as the pigments of mollusc shells and the eggs and feathers of certain birds.

Heme proteins may be soluble—for example, hemoglobin, catalase, tryptophan pyrrolase—or they may be bound to cellular membranes, in which case they are termed "cytochromes." Cytochromes function as terminal oxidases—for example, mitochondrial cytochromes a, endoplasmic reticulum bound cytochromes b_5 and P-450; or as intermediates in electron transfer chains such as mitochondrial cytochromes b and c. Cellular respiration, energy generation, and chemical oxidations are dependent

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