Reports

Isotopic Composition of Cellulose from C_3, C_4, and CAM Plants Growing Near One Another

Abstract. *Cellulose from plants having crassulacean acid metabolism was enriched in deuterium but not in oxygen-18 in relation to cellulose from C_3 and C_4 plants growing in the same area, indicating that the deuterium enrichment is due to isotopic fractionation during biochemical reactions rather than during evapotranspiration. Hydrogen and oxygen stable isotope ratios of cellulose from the plants in this restricted area showed more variability than that observed in samples collected across an entire continent. Biological factors appear to be as important as environmental factors in determining the isotope ratios of plant cellulose.*

Contradictory observations on the hydrogen isotope ratios of plants operating under different photosynthetic modes have been reported. Ziegler *et al.* (*1*) found that C_3, C_4, and CAM (crassulacean acid metabolism) plants (*2*) grown in a greenhouse as well as plants collected in the wild could be distinguished from one another based on their hydrogen isotope ratios. On the other hand, Smith and Epstein (*3*) observed only slight differences in hydrogen isotope ratios of salt-marsh plant species representing the three different photosynthetic pathways. Interpretation of these observations is complicated by several factors. First, most of the measurements of hydrogen isotope ratios were done on total organic matter and since different chemical components of plant matter can have different isotope ratios (*4*), differences in chemical composition between plants might contribute to the reported isotopic differences. Furthermore, a large portion of organically bound hydrogen is exchangeable with water (*4*) and thus procedures such as cleaning the sample by washing it in water might change its isotopic value. Another factor is that the plants analyzed in one study (*1*) were collected over a wide geographical area. Hydrogen isotope ratios in the meteoric water available to plants varies with geographical location (*5*). Climatic factors also influence the hydrogen isotopic composition of water available for photosynthesis (*6, 7*). Thus the reported differences (*1*) in hydrogen isotope ratios of C_3, C_4, and CAM plants might be due in part to differences in the climate or isotope ratios of the meteoric waters to which they were exposed.

We sampled C_3, C_4, and CAM plants from a limited geographic area to minimize some of the complicating factors, and we extracted cellulose for isotopic analysis, thus eliminating the possibility that differences in isotope ratios of different plants were due to differences in chemical composition. Furthermore, we analyzed cellulose nitrate in order to measure only the isotope ratios of nonexchangeable carbon-bound hydrogen (*4*).

Photosynthetic mode, physiognomy, location of sampling, $\delta^{18}O$ values of cellulose, and $\delta^{13}C$ and δD values (*8*) of cellulose nitrate of the plants are given in Table 1. The δD values are plotted against the $\delta^{13}C$ values in Fig. 1A. The $\delta^{13}C$ values of C_3, C_4, and CAM plants were similar to values reported previously for plants using these photosynthetic pathways (*9*). The δD values of CAM plants ranged from about +30 to +80 per mil, distinctly higher than those of C_3 and C_4 plants, which had δD values ranging from about −140 to −20 per mil. These results agree in part with those reported by Ziegler *et al.* (*1*), in that CAM plants were enriched in deuterium relative to C_3 and C_4 plants, but the differences we observed between C_3 and C_4 plants were smaller. Analysis of more C_3 and C_4 plants from the same locality is needed to determine whether this difference is significant.

Ziegler *et al.* (*1*) suggested that CAM plants have more positive δD values than C_3 and C_4 plants because of their ability to maintain metabolic activity during periods of water stress. They proposed that during such periods, deuterium enrichment of plant water due to evapotranspiration and subsequent labeling of organically bound hydrogen occur. Two lines of evidence argue against this explanation. First, CAM plants transpire only during nights and therefore undergo less evapotranspiration than C_3 and C_4 plants, which transpire during warmer, drier days (*10*). Evapotranspiration causes an increase in hydrogen isotope ratios of leaf water relative to meteoric water (*6, 7, 11, 12*). Thus one would expect the δD values of C_3 and C_4 plants to be higher than those of CAM plants. In fact, what we and Ziegler *et al.* (*1*) observed was the opposite. Second, if the explanation of Ziegler *et al.* (*1*) is correct, then the cellulose $\delta^{18}O$ values of CAM plants should also be higher than those of C_3 and C_4 plants, since evapotranspiration also causes enrichment of ^{18}O in plant water (*11, 13*) and consequently in cellulose (*12*). This is not what we observed (Fig. 1B), suggesting that the evapotranspiration hypothesis (*1*)

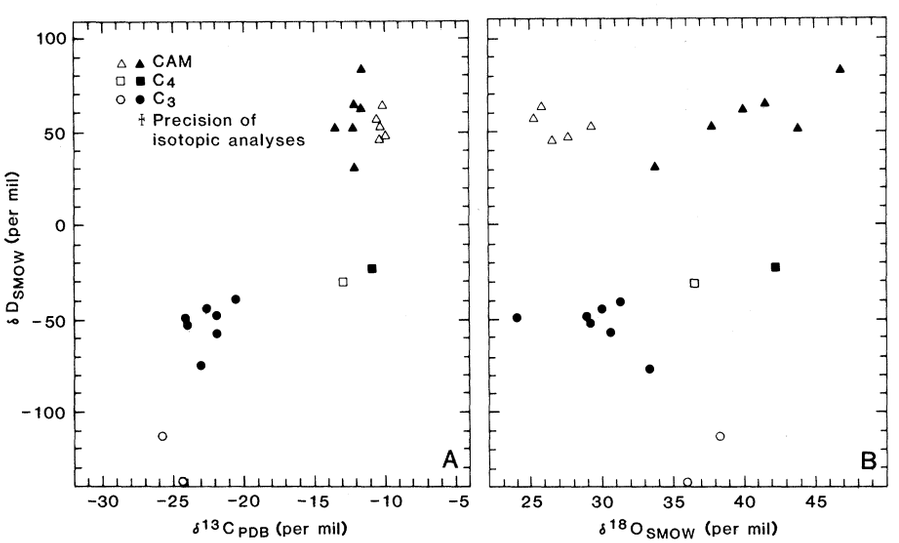

Fig. 1. Relation (A) between the δD and $\delta^{13}C$ values of cellulose nitrate and (B) between the δD values of cellulose nitrate and $\delta^{18}O$ of cellulose for plant species having the indicated photosynthetic modes. The physiognomy of the plants is indicated as follows: stem succulent (▲), leaf succulent (△), herbaceous annual (■), herbaceous perennial (□), woody perennial (●), and grass (○).

does not explain the deuterium enrichment of CAM plants. The broad overlap that we observed in the oxygen isotope ratios among plants having different photosynthetic pathways, without an overlap in the hydrogen isotope ratios between CAM plants and C_3 and C_4 plants, suggests that isotopic fractionations during biochemical reactions are responsible for the hydrogen isotope differences between CAM plants and C_3 and C_4 plants. Our understanding of isotopic fractionation of hydrogen in plants is too rudimentary to propose a model for such biochemical fractionations.

Epstein *et al.* (*7*) have also determined both the $\delta^{18}O$ and δD values of cellulose and cellulose nitrate from terrestrial plants. They analyzed C_3 plants collected throughout the North American continent from a variety of environments, ranging from a semitropical island to arctic tundra. In the continental sample the δD values of different plants differed by as much as 199 per mil and the $\delta^{18}O$ values by as much as 19 per mil. Within our restricted collection area, the δD values of different plants ranged over 220 per mil and the $\delta^{18}O$ values differed by as much as 22 per mil. Although the larger amount of variability in isotope ratios that we observed in plants from a limited collection area may be due to the fact that these plants use all three photosynthetic pathways, our results have implications for the use of isotope ratios of cellulose in reconstructing paleoclimates.

A good correlation was reported between the hydrogen isotope ratios of cellulose nitrate and the meteoric water available to the plants collected throughout the North American continent (*4*). Thus it was proposed that the δD values of the meteoric water to which a plant was exposed could be estimated by adding 22 per mil to the δD value of cellulose nitrate, and, since the δD values of the meteoric water are related to temperature (*5*), the hydrogen isotope ratios from cellulose nitrate prepared from fossil plants could be used to estimate temperatures of ancient climates (*4*). Within each physiognomic group in our sample, hydrogen isotope ratios were fairly constant. However, isotope ratios differed by large amounts when different physiognomic groups or plants with different photosynthetic pathways were considered. Using δD values of cellulose nitrate from C_3 grasses, C_3 woody perennials, or CAM plants, one would calculate different isotope ratios of meteoric water by the relationship stated above. Biological factors such as physiognomy and photosynthetic mode thus will complicate the determination of isotope ratios of meteoric water from cellulose nitrate δD values.

Variations in oxygen isotope ratios of cellulose have been ascribed to differences in the isotopic composition of meteoric water as well as to differences in climate at the collection sites (*7, 14, 15*). Our results for plants collected in a restricted area, where variations in isotopic composition of meteoric water and climate were minimal, suggest that biological factors may also be important in determining the $\delta^{18}O$ values of plant cellulose. The $\delta^{18}O$ values appear to be related to physiognomic characteristics rather than photosynthetic pathways (Fig. 1B). If this is the case, $\delta^{18}O$ variability would be due to differences in evapotranspiration between plant species rather than to differences in biochemistry.

Our analysis of plants operating in the three photosynthetic modes in a restricted environment showed that cellulose nitrate δD values of CAM plants are higher than those of C_3 and C_4 plants. Analysis of oxygen isotope ratios from these plants indicates that the deuterium enrichment results from isotopic fractionations occurring during biochemical reactions rather than during evapotranspiration. Analysis of a more diverse group of plants should add to our understanding of the biochemical reactions that lead to distinct δD values in CAM plants, particularly analysis of plants with differing proportions of C_3 metabolism and CAM (*10*). The relation between the δD and $\delta^{13}C$ values of cellulose nitrate from these plants should indicate the way in which δD values in CAM plants are related to their own particular type of carbon metabolism. Analysis of submerged aquatic CAM plants (*16*) should elucidate the isotopic fractionations that occur during biochemical reactions in CAM plants independent of evapotranspiration.

Finally, because physiognomic characteristics and photosynthetic mode seem to be as important as environmental factors in influencing the isotope ratios of plant cellulose, an understanding of these biological factors should be a prerequisite to climate reconstruction based on isotopic analysis of cellulose from different photosynthetic and physiognomic types of plants.

Table 1. Photosynthetic mode, physiognomy, site of collection, $\delta^{18}O$ values of cellulose, and δD and $\delta^{13}C$ values of cellulose nitrate (*8*) of different plant species collected at the Philip L. Boyd Deep Canyon Desert Research Center in Riverside County, California. Most plants were collected within 3 km of one another at 300 m above sea level. The rest were collected within 100 m of one another at a site 850 m above sea level and about 2 km away from the first site. Several leaves from the *Agave deserti* specimens were analyzed. Samples were dried for 24 hours at 50°C, vacuum-dried for another 24 hours, then ground into a powder in a Wiley mill. Cellulose and cellulose nitrate were prepared by methods described previously (*17*). Cellulose oxygen isotope ratios were determined by a modification of the Rittenberg and Ponticorvo method (*18*). Cellulose nitrate carbon and hydrogen isotope ratios were determined as described elsewhere (*19*). Abbreviations: WP, woody perennial; Gr, grass; SS, stem succulent; LS, leaf succulent; HA, herbaceous annual; and HP, herbaceous perennial.

Species	Photosynthetic mode	Physiognomy	Site	$\delta^{18}O_{SMOW}$ (per mil)	δD_{SMOW} (per mil)	$\delta^{13}C_{PDB}$ (per mil)
Cercidium floridum	C_3	WP	Lower	+28.9	−48	−21.9
Simmondsia chinensis	C_3	WP	Lower	+30.7	−57	−21.8
Hyptis emoryi	C_3	WP	Lower	+29.2	−53	−24.0
Parosela spinosa	C_3	WP	Lower	+30.0	−46	−22.7
Larrea tridentata	C_3	WP	Lower	+31.4	−40	−20.5
Larrea tridentata	C_3	WP	Upper	+23.9	−50	−24.2
Hymenoclea salsola	C_3	WP	Lower	+33.4	−77	−23.1
Schismus barbatus	C_3	Gr	Lower	+36.0	−140	−24.3
Bromus rubens	C_3	Gr	Lower	+38.3	−114	−25.8
Opuntia ramosissima	CAM	SS	Lower	+41.6	+65	−12.2
Opuntia bigelovii	CAM	SS	Lower	+46.6	+83	−11.8
Opuntia acanthocarpa	CAM	SS	Lower	+43.8	+52	−12.2
Opuntia basilaris	CAM	SS	Lower	+33.8	+32	−12.3
Ferocactus acanthodes	CAM	SS	Lower	+37.8	+53	−13.6
Echinocereus engelmannii	CAM	SS	Lower	+40.2	+63	−11.9
Agave deserti	CAM	LS	Lower	+25.9	+64	−10.3
Agave deserti	CAM	LS	Lower	+25.2	+56	−10.7
Agave deserti	CAM	LS	Upper	+27.6	+47	−10.0
Agave deserti	CAM	LS	Upper	+29.3	+54	−10.5
Agave deserti	CAM	LS	Upper	+26.5	+46	−10.6
Salsola iberica	C_4	HA	Lower	+42.2	−24	−11.0
Euphorbia polycarpa	C_4	HP	Lower	+36.5	−30	−13.3

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2. In C_3 plants, carbon dioxide is fixed into a three-carbon sugar. This fixation and the subsequent metabolic cycle is known as the Calvin cycle. C_4 plants fix carbon dioxide into a four-carbon compound such as malic or aspartic acid, which is subsequently decarboxylated. The carbon dioxide then enters the Calvin cycle as in a C_3 plant. Stomatal opening and carbon dioxide uptake occur during the day in C_3 and C_4 plants. In contrast, carbon dioxide is fixed into malic acid during the night in CAM plants. The malic acid is then decarboxylated during the day and the carbon dioxide enters the Calvin cycle as in a C_3 plant.
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8. The δ values are defined as

$$[(R_{sample}/R_{standard}) - 1] \times 1000$$

where $R = {}^{18}O/{}^{16}O$ for $\delta^{18}O$, R = deuterium/hydrogen for δD, and $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$. The standard is standard mean ocean water (SMOW) for hydrogen and oxygen and the Peedee belemnite (PDB) for carbon.
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Total Synthesis of the L-Hexoses

Abstract. *Enantiomerically pure polyhydroxylated natural products are synthesized by using a reiterative two-carbon extension cycle consisting of four steps. The generality and efficiency of this methodology are demonstrated in the total synthesis of all eight L-hexoses.*

We describe here the systematic, stereoselective synthesis of all eight L-hexoses by a synthetic methodology developed in our laboratories for the preparation of polyhydroxylated natural products (*1*). Most monosaccharide syntheses have involved modification of sugars that occur naturally (*2*), and recorded total syntheses have usually been carried out in a racemic form and with poor stereoselection (*3*). The stereochemical challenge involved in a general synthesis of monosaccharides, though purely academic, has now been met, and a high degree of stereocontrol is attainable (*4–6*).

Our strategy is based on the reiterative two-carbon extension cycle, which consists of four steps (Fig. 1): I, conversion of an aldehyde into its corresponding *E*-allylic alcohol; II, asymmetric epoxidation (AE) with titanium tetraisopropoxide, *t*-butylhydroperoxide, and diethyl (+)- or (−)-tartrate; III, treatment of the epoxy alcohol with benzenethiolate anion in a basic medium; and IV, oxidation and Pummerer reaction of the sulfide followed by the net hydrolysis of the resulting *gem*-acetoxysulfide with or without inversion of the C(2) center (*7*). Because of the presence of four hydroxymethylene centers in the hexoses, the synthesis of these compounds requires a double application of the basic cycle.

The synthesis described here begins with a single fundamental building block, 4-benzhydryloxy-(*E*)-but-2-en-1-ol, **1**, a compound which is readily prepared from (*Z*)-2-butene-1,4-diol (*8*). Step I of the extension cycle is therefore eliminated in this initial case. The selection of the benzhydryl protecting group rather than a more common group such as benzyl has proved to be critical (see below), and the benzhydryl serves its purpose through the entire synthesis, which is shown in Fig. 2 with the yield and selectivity for each step. Conversion of **1** into **4** and **5** completes the first cycle, and the conversion of **4** and **5** into **16** to **23** constitutes the entire set of the second cycle. A succinct explanation of Fig. 2 with comments is given below with the routes leading to L-allose (**16**) and L-altrose (**17**) as examples.

(Conversion of **1** → **4**) Sulfide **3** is prepared in the manner described for the case of R = benzyl (*5*). Oxidation of **3** to the sulfoxide followed by a Pummerer rearrangement under Tsuchihashi conditions (*9, 10*) provides the *gem*-acetoxysulfide intermediate, which is reduced with Dibal at −78°C to give the aldehyde **4** in overall 84 percent yield. The epimeric aldehyde **5** is absent. (**4** → **6b**) Homologation of **4** with formylmethylenetriphenylphosphorane followed by reduction with sodium borohydride leads to allylic alcohol **6b** in 88 percent yield and with excellent selectivity. This compound is ready for application of the second AE. (**6b** → **8**) In earlier experiments in which the substrate **6b′** was used with R = benzyl, the AE reaction was accompanied by a subsequent titanium-catalyzed epoxide opening. This process involved participation by the oxygen atom of the C(6) benzyloxy group, which led to formation of the undesired corresponding tetrahydrofuran. Use of **6b**, however, prevents this ethereal oxygen participation and AE proceeds smoothly. The selectivity of this reaction appears perfect, as no trace of the diastereoisomer **9** is detected by the usual techniques of analysis. (**8** → **16**) Treatment of epoxy alcohol **8** with benzenethiolate anion in an alkaline medium results in the isolation of a diol (77 percent), which is quantitatively transformed into **12**. A sequence of high-yielding steps—(i) oxidation and Pummerer rearrangement (90 percent) and (ii) Dibal reduction (81 percent), deprotection with trifluoroacetic acid, and catalytic hydrogenation (90 percent)—affords the free sugar L-allose (**16**). Alternatively, potassium carbonate in metha-

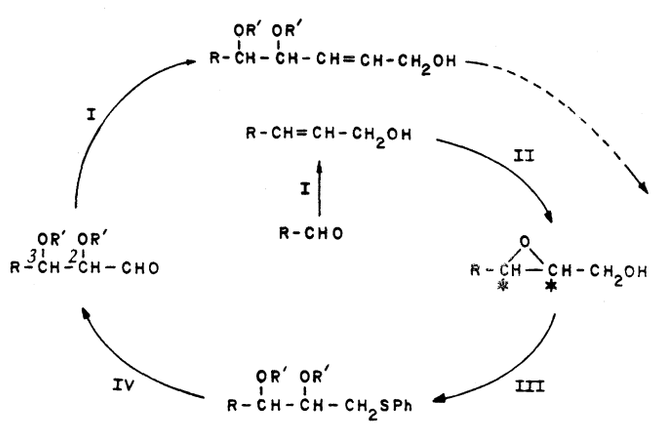

Fig. 1. Reiterative two-carbon extension cycle.