Phytochemistry, Vol 23, No 11, pp 2475–2477, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

STABLE HYDROGEN ISOTOPE RATIOS OF SAPONIFIABLE LIPIDS AND CELLULOSE NITRATE FROM CAM, C_3 AND C_4 PLANTS

LEONEL STERNBERG, MICHAEL J DENIRO and HENRY AJIE

Department of Earth and Space Sciences, University of California, Los Angeles, CA 90024, U S A

(*Revised received* 12 *April* 1984)

Key Word Index—Angiospermae, hydrogen isotope ratios, saponifiable lipid, cellulose nitrate, deuterium, protium, photosynthesis

Abstract—Hydrogen and carbon isotope ratios of saponifiable lipids and cellulose nitrate from CAM, C_3, and C_4 plants that grew near one another were determined The deuterium/protium (D/H) ratios of cellulose nitrate from CAM plants were much higher than those of cellulose nitrate from C_3 and C_4 plants, as has been observed previously In contrast, the D/H ratios of saponifiable lipids from CAM plants did not differ from those of the same fraction from C_3 and C_4 plants These observations indicate that deuterium enrichment in cellulose of CAM plants is not caused by any metabolic or physiological process which would lead to deuterium enrichment in all biochemical fractions

INTRODUCTION

A considerable body of research done during the past two decades has demonstrated that carbon isotope ratios of plant matter differ for plants having different photosynthetic modes [1–3] More recently, it has been shown that hydrogen isotope ratios of whole plant matter and of cellulose nitrate also depend on photosynthetic modes [4–8] δD values (see Experimental for the definition of δ values) of cellulose nitrate from CAM plants are about 100‰ greater than the δD values of cellulose nitrate from C_3 and C_4 plants that grew nearby [6–8]

There are several hypotheses that could explain the deuterium enrichment of cellulose from CAM plants Amongst these, two that have been proposed predict a proportional deuterium enrichment for all metabolic fractions in CAM plants relative to the same fractions in C_3 and C_4 plants [4] The first hypothesis, proposed by Ziegler *et al* [4], involves the idea that the deuterium enrichment in CAM plants is caused by deuterium enrichment of plant water that occurs during evapotranspiration and the subsequent labeling of the organic hydrogen by this enriched water [4] In the same paper, Ziegler *et al* [4] proposed that enzymes associated with photochemical production of NADPH in C_4 plants could show hydrogen isotope effects different than those in C_3 plants This hypothesis might also serve as an explanation for the deuterium enrichment in CAM plants The CAM chloroplast might produce NADPH enriched in deuterium which would eventually label the organically bound hydrogen of carbohydrates If either of the two hypotheses is true, then all biochemical fractions of CAM plants should show deuterium enrichment relative to the corresponding fractions from C_3 and C_4 plants This follows from two observations the water in the plant is the ultimate source of the hydrogen available for cellular biosynthesis, and hydrogen for all biochemical fractions comes from photosynthetically produced NADPH, since all metabolites in plants originate from carbohydrates Other hypotheses, which explain deuterium enrichment in CAM plants as being based on the segregation of source metabolites into separate pools with different extents of deuterium enrichment, would predict different isotope ratios for different metabolites

In this paper we report the analysis of hydrogen isotope ratios of two different biochemical fractions, lipids and cellulose, from CAM, C_3 and C_4 plants The results of our study eliminate hypotheses which predict that all biochemical fractions of CAM plants are enriched in deuterium relative to the same fractions from C_3 and C_4 plants

RESULTS

δD values were determined for cellulose nitrate to eliminate the possibility that differences in isotope ratios between different plants are due to differing proportions of biochemical fractions or amounts of exchangeable (non-carbon-bound) hydrogen between species [9] Almost all hydrogen from saponifiable lipids are non-exchangeable since they are carbon-bound, so that there are virtually no problems associated with measurement of exchangeable hydrogens in this fraction [10]

δD values of cellulose nitrate and saponifiable lipids for plants having the three photosynthetic modes (C_3, C_4 and CAM) are shown in Fig 1 Some of the cellulose nitrate data have been reported previously [7] The δD values of cellulose nitrate of CAM plants were about 100‰ higher than those of C_3 and C_4 plants In contrast, the saponifiable lipids from CAM plants were not enriched in deuterium relative to saponifiable lipids of C_3 and C_4 plants In all cases, the δD values of the lipids were lower than the δD values of the cellulose nitrate, as has been previously reported [9, 10]

δ^{13}C values of cellulose nitrate and saponifiable lipid of C_3, C_4 and CAM plants are shown in Fig 2 δ^{13}C values of saponifiable lipids for all plants were more negative than the values observed for cellulose nitrate, as has been reported previously [9, 10]

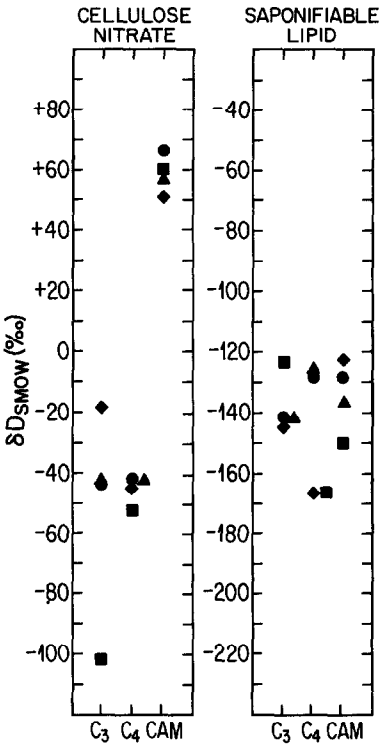

Fig 1 δD values of cellulose nitrate and saponifiable lipids of C_3, C_4 and CAM plants Note sliding of the two δD scales The C_3 plants are *Acacia rigidula* (◆), *Lippia graveolens* (▲), *Penstemon bacharifolius* (■) and *Prosopis glandulosa* (●) The C_4 plants are *Aristidia wrightii* (■), *Bouteloua hirsuta* (▲), *Hateropogon contortus* (◆), and *Pappophorum bicolor* (●) The CAM plants are *Echinocereus enneacanthus* (■), *Ferocactus hamataeanthus* (◆), *Opuntia leptocaulis* (▲), and *Yucca baccata* (●)

DISCUSSION

The δD values of saponifiable lipids from CAM plants are not substantially different from those observed for saponifiable lipids of C_3 and C_4 plants, yet there are large differences in the δD values between the cellulose nitrate

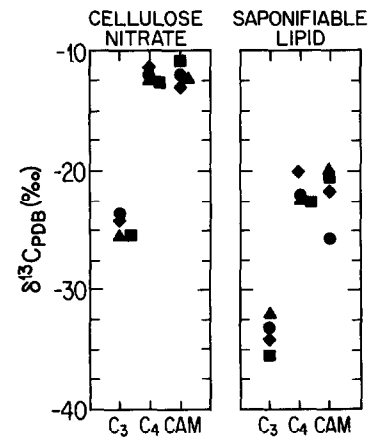

Fig 2 The δ^{13}C values of cellulose nitrate and saponifiable lipids of C_3, C_4 and CAM plants The symbols are the same as those used in Fig 1

fractions of CAM plants and of C_3 and C_4 plants Analysis of hydrogen isotope ratios in total lipids and in cellulose nitrate from one CAM, one C_3 and C_4 plant species have been reported previously [5] The differences in δD values of lipids from the three photosynthetic modes observed by these workers were well within the variability we observed within each photosynthetic mode, and thus no conclusions can be drawn from their observations

Our results eliminate two hypotheses, discussed above, which have been advanced to account for deuterium enrichment in CAM plants CAM plants do not have elevated δD values because of deuterium enrichment in plant water caused by evapotranspiration, because such enrichment would also cause a relative deuterium enrichment in the lipid fraction We have previously reached the same conclusion based on the relationship between the hydrogen isotope ratios of cellulose nitrate and the oxygen isotope ratios of cellulose [6] Deuterium-enriched NADPH produced by the CAM chloroplast would also cause a proportional enrichment in the lipid fraction We did not observe this proportional enrichment in the lipids from CAM plants relative to the lipids of C_3 and C_4 plants

Although our knowledge of the basis for hydrogen isotope fractionation in plants is rudimentary, our data suggest that deuterium enrichment in cellulose from CAM plants originates from a deuterium-enriched fraction which goes on to cellulose synthesis Further, the deuterium enrichment of cellulose in CAM plants does not occur by some basic process which would affect all biochemical components Any hypothesis that explains the deuterium enrichment of cellulose from CAM plants must account for the data presented here

We favor the following model Cellulose in CAM plants comes from a deuterium-enriched carbohydrate pool while the lipids are derived from a separate deuterium-depleted carbohydrate pool The lipids are derived from a carbohydrate pool which comes directly from the Calvin cycle in all three photosynthetic types In CAM plants the carbohydrate pool which provides PEP for carboxylation and is regenerated via gluconeogenesis of pyruvate after decarboxylation of malic acid [11, 12] is the pool of carbohydrate available for cellulose synthesis Further, the cycle of glycolysis to PEP and gluconeogenesis from pyruvate enriches this carbohydrate pool in deuterium C_4 plants do not have this deuterium enrichment of carbohyrates because pyruvate, the product of the decarboxylation, is reused for carbon fixation and does not enter the gluconeogenic cycle

There are several aspects of this model which need to be tested However, there are two observations which are consistent with it The first is the observation of Deleens and her coworkers [11, 12] that there are two distinct carbohydrate pools in *Kalanchoe blossfeldiana* and *Bryophyllum daigremontianum*, both of which are CAM plants One carbohydrate pool is associated with a starch–malate sequence and the other is associated with the carbohydrate produced during the Calvin cycle Our hypothesis departs from the conclusions of Deleens *et al* [11] in that we propose that cellulose is synthesized from carbohydrates of the starch–malate pool rather than from the carbohydrate pool generated by the Calvin cycle The other finding which supports our model is the observation of Estep and Hoering [13] that the δD value of total organic matter for *Chlorella* fed on the acetate is higher than that of acetate by about 50‰ This suggests that the

gluconeogenic cycle from acetate to carbohydrate and other metabolites is a deuterium enriching process It must be shown that deuterium enrichment also occurs during gluconeogenesis from pyruvate to carbohydrate Further, it would be important in the context of the findings presented here to demonstrate that such enrichment extends to the non-exchangeable hydrogen of the carbohydrate fractions

One other hypothesis that is not inconsistent with the results presented here has been proposed Ziegler *et al* [4] suggested that NADPH generated by the malic enzyme reaction during decarboxylation of malic acid is compartmentalized, enriched in deuterium and used in cellulose synthesis Previously published results [8], although inconclusive, suggest that CAM plants which have a decarboxylation pathway alternate to the malic enzyme pathway (namely the PEP-carboxykinase reaction) [14] still have elevated cellulose nitrate δD values relative to C_3 and C_4 plants Thus this hypothesis may not be correct

Whatever causes deuterium enrichment in CAM plants, the results presented here considerably narrow the choice of models that can account for the phenomenon

EXPERIMENTAL

Samples were collected within 200 meters of one another in the Pecos River area (Val Verde County, Texas) as reported by Sternberg *et al* [7] Samples were dried at 50°, then ground into a fine powder in a Wiley mill Cellulose was prepared by the method of Wise [15] and nitrated by the acetic anhydride method as in DeNiro [16] Lipids were extracted and saponified by the method described in Northfelt *et al* [10] Carbon and hydrogen isotope ratios of cellulose nitrate and saponifiable lipids were determined by a modified version of the Stump and Frazer method [10, 17] Isotope ratios are expressed as δ values where

$$\delta(‰) = \left[\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right] \times 1000$$

and R represents D/H for hydrogen and $^{13}C/^{12}C$ for carbon The standards were standard mean ocean water (SMOW) for hydrogen and the Peedee belemnite (PDB) carbonate for carbon The precisions of isotopic analysis were $\pm 2‰$ for δD values and $\pm 0.2‰$ for $\delta^{13}C$ values

REFERENCES

1 Bender, M M (1971) *Phytochemistry* **10**, 1239
2 Bender, M M, Vines, H M and Black, C C (1973) *Plant Physiol* **52**, 427
3 Smith, B N and Epstein, S (1970) *Plant Physiol* **47**, 380
4 Zeigler, H, Osmond, C B, Stickler, W and Trimborn, P (1976) *Planta* **128**, 85
5 Ziegler, H (1979) *Ber Deutsch Bot Ges Bd* **92**, 169
6 Sternberg, L and DeNiro, M J (1983) *Science* **220**, 947
7 Sternberg, L, DeNiro, M J and Johnson, H B (1984) *Plant Physiol* **74**, 557
8 Sternberg, L, DeNiro, M J and Ting, I P (1984) *Plant Physiol* **74**, 104
9 Epstein, S, Yapp, C J and Hall, J H (1976) *Earth Planet Sci Letters* **30**, 241
10 Northfelt, D W, DeNiro, M J and Epstein, S (1981) *Geochim Cosmochim Acta* **45**, 1895
11 Deleens, E, Garnier-Dardart, J and Queiroz, O (1979) *Planta* **146**, 441
12 Deleens, E, Garnier-Dardart, J and Queiroz, O (1981) in *Photosynthesis IV* (Royunoglou, G A, ed) p 561 Balaban International Science Services, Philadelphia
13 Estep, M F and Hoering, T C (1980) *Geochim Cosmochim Acta* **44**, 1197
14 Dittrich, P, Campbell, W H and Black, C C (1973) *Plant Physiol* **52**, 357
15 Wise, L E (1944) *Wood Chemistry* Reinhold, New York
16 DeNiro, M J (1978) *Earth Planet Sci Letters* **54**, 177
17 Stump, R K and Frazer, J W (1973) *Nucl Sci Abstr* **28**, 746