Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Organic Geochemistry 34 (2003) 413–423
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Preservation of lipid hydrogen isotope ratios in Miocene lacustrine sediments and plant fossils at Clarkia, northern Idaho, USA

Hong Yang[a,b,*], Yongsong Huang[b]

[a]*Department of Science and Technology, Bryant College, Smithfield, RI 02917, USA*
[b]*Department of Geological Sciences, Brown University, Providence, RI 02912, USA*

Received 9 March 2002; accepted 15 October 2002
(returned to author for revision 4 July 2002)

Abstract

We measured D/H ratios of individual lipids isolated from plant fossils and water-lain sediments from the Miocene Clarkia lacustrine deposit (15–20 Ma) in northern Idaho, USA, in order to assess the preservation potential of lipid hydrogen isotope ratios. Distinct *n*-alkyl lipid profiles in plant fossils (*Platanus, Quercus*, *Salix*) and the sedimentary matrix demonstrate the high degree of lipid preservation, as previously reported. Our data suggest that original hydrogen isotope ratios of carbon-bound hydrogen in lipids may have been preserved after 15–20 million years of burial in the water-lain lacustrine sediment, because δD values of individual lipids show large variations between different fossil genera and between fossils and sediments, and hydrogen isotope values of fossil lipids differ by more than 123‰ from the associated sediment water. These data are consistent with minimal isotopic exchange between carbon-bound hydrogen in lipids and sediment water hydrogen. Our results validate the use of lipid hydrogen isotope ratios for paleoecological studies over an extended geological time period.

1. Introduction

The large isotopic effects for hydrogen and the large deuterium/hydrogen (D/H) variations known to exist in nature make hydrogen isotopes an attractive candidate for geological applications (Sessions et al., 1999; Ward et al., 2000; Sessions, 2001). However, the small quantity of naturally occurring deuterium poses an analytical challenge for accurate measurement of δD in geological samples. Compared with ^{13}C abundance in natural samples (1.1%), deuterium has a mean natural abundance of only 0.015% (Hilkert et al., 1999). Recent technological breakthroughs in gas chromatography/thermal conversion/isotope ratio mass spectrometry (GC/TC/IRMS) (Burgoyne and Hayes, 1998; Hilkert et al., 1999) made it possible to measure precisely compound-specific hydrogen isotopes in sediments and fossils, and opened the possibility of applying the technique to geological samples in small quantities. However, to date, studies involving compound specific D/H ratio of ancient lipid biomarkers are largely limited to Holocene samples (Xie et al., 2000; Sauer et al., 2001), with only one report suggesting that original hydrogen isotope ratios may be preserved in 6 million year old sediments (Andersen et al., 2001). There is a major concern regarding significant hydrogen isotope exchange between preserved organic molecules and the surrounding environment during diagenesis over an extended geological time scale. Recent laboratory biodegradation experiments highlighted the potential of H isotopic variations in sedimentary organic molecules (Ward et

* Corresponding author. Tel.: +1-401-232-6223; fax: +1-401-232-6492.
E-mail address: hyang@bryant.edu (H. Yang).

0146-6380/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0146-6380(02)00212-7

al., 2000; Hunkeler et al., 2001; Pond et al., 2002). In an incubation experiment using D_2O, Sessions (2001) reported that deuterium incorporation from heavy water into incubated organic molecules has occurred rapidly, and that the D/H exchange was enhanced by the presence of clay minerals in acidic, organic-rich lacustrine sediments. The experimental results of Sessions (2001) are inconsistent with field observations from sediment studies (Xie et al., 2000; Sauer et al., 2001; Andersen et al., 2001) that detected primary D/H ratios. Thus, whether older fossil material preserved in sedimentary deposits can retain primary D/H compositions in their lipid biomarkers, and to what extent compound-specific hydrogen isotope analysis can be applied to paleoecological and paleoenvironmental studies at the geological time scale are of considerable interest to the geological community.

The Clarkia Miocene lacustrine deposit (15–20 million years old) at the St Maries River Valley in northern Idaho, USA (Smiley et al., 1975; Smiley and Rember 1985; Yang, 1993; Yang et al., 1995) has been a treasure-trove for studying molecular preservation. A variety of ancient biomolecules and different classes of organic compounds have been reported from the deposit (Giannasi and Niklas, 1981; Niklas et al., 1985; Rieseberg and Soltis, 1987; Golenberg et al., 1990; Soltis et al., 1992; Logan et al., 1993). Previous studies demonstrated that lipid compounds were well-preserved both in fossil leaf compressions (Logan et al., 1993; Lockheart et al., 2000) and water-lain lake sediments (Logan and Eglinton, 1994). Leaf waxes were found to still be localized inside the leaf tissues after more than 15 million years of burial (Logan et al., 1995; Huang et al., 1995, 1996). In addition, previous measurements of molecular ^{13}C from Clarkia plant fossils indicated preservation of compound-specific carbon isotope signatures in Clarkia material (Huang et al., 1995, 1996).

Several aspects of the Clarkia deposit made it an ideal site for examining δD preservation and possible D/H exchange in natural conditions. Firstly, the Clarkia sediments and fossils are water logged, making it a natural laboratory for testing possible deuterium-hydrogen exchange between preserved lipids and the surrounding water. Secondly, the Clarkia sediments are rich in organic matter (5.5% TOC, Logan and Eglinton, 1994), and the associated water in sediments and fossils is acidic (pH = 5.5). Thirdly, the Clarkia sediments contain abundant clay minerals. An estimated 20–30% clay minerals were observed under transmission light microscopy (Richard Yund, personal communication).

The primary objective of this paper is to evaluate the degree of preservation of lipid hydrogen isotope ratios in the 15–20 million year old Clarkia sediments and fossils. We attempt to accomplish this goal by measuring the hydrogen isotopic compositions of individual lipids in three different plant fossils, their modern counterparts, and sediments bearing the plant fossils. We also measure the δD values of sediment water and modern environmental water for comparison. If lipid hydrogen rapidly exchanged with the environmental hydrogen, we would expect to find relatively homogenous δD values for compounds of similar structures in ancient samples and a diminished offset between fossil lipid δD values and sediment water after 15–20 million years of isotopic exchange.

2. Samples and methods

2.1. Fossil and sediment samples

Large blocks (normally 30 cm/20 cm/10 cm, L/W/H) of fossiliferous siltstones were collected from the outcrop of lacustrine phase Horizon 2B at the P-33 site in Clarkia (Smiley and Rember, 1985), and were shipped to the laboratory on ice until analyzed. In the laboratory, the rocks were split open along laminations to expose fossil materials. Leaf compressions of three genera of higher plants, *Platanus*, *Quercus*, and *Salix*, were identified based on gross morphological characters. The three genera were selected for this study because higher abundance of lipids and thicker cuticles were known from fossil *Platanus* and *Quercus* (Logan et al., 1995; Huang et al., 1995; Lockheart et al., 2000). No fossil *Salix* lipid was previously examined. Leaf tissues of 0.25–1 g were immediately removed or scraped into a vial using solvent-washed knife, and care was taken to keep associated sedimentary matrix to a minimum. Two samples of fine sediment powder were obtained by scraping the portion of sedimentary rock where no macrofossil was visible.

2.2. Living plant leaves

For comparison purposes, leaves of three living plant species, American sycamore (*Platanus occidentalis* L.), Oregon white oak (*Quercus garryana* Dougl.), and peachleaf willow (*Salix amygdaloides* Anderss.), were collected from Idaho. The willow leaves were sampled from Clarkia, only 300 m away from the P-33 fossil site, whereas the sycamore and oak specimens were collected from the University of Idaho campus at Moscow, 85 km from Clarkia. To avoid cross contamination between modern and fossil molecules, the modern samples were analyzed after the analysis of fossil specimens was completed.

2.3. Water samples

Both sediment waters that are now associated with Miocene fossils at the Horizon 2B and surface waters near the P-33 site and from Moscow were collected. The sediment waters were collected in the following two different

ways. In the field, water seeped off from sedimentary blocks were immediately pipetted from the rock and stored in a vial. The same water-lain sedimentary blocks were sealed into plastic bags, and water is condensed at room temperature. The water condensed within the bag was pipetted out for analysis. Surface waters were sampled during a 3-day period in August 2001 from the Clarkia Creek near P-33 site and from a pond 500 m away from the growing site of the peachleaf willow. Surface waters were also collected, during the same time period, from Moscow, Idaho near the growth site of American sycamore and Oregon white oak from which leaves were obtained.

2.4. Lipid extraction and separation

Methods for extraction, fractionation, and purification of fossil lipids were similar to previously described (Logan and Eglinton, 1994; Huang et al., 1995). Total lipid extraction was carried out with Dionex 200 Accelerated Solvent Extractor (ASE) using 2:1(v/v) dichloromethane(DCM):methanol solvent mixture. Lipids from modern leaves were extracted by dipping pre-washed leaves into DCM for 2 min. Solid phase ion exchange columns (3 ml—Supelco) were used to separate free carboxylic acids from the neutral lipid fractions, which were further separated into hydrocarbons and alkanols by silica gel flash column chromatography using solvents of increasing polarity. The acid fraction was methylated using 2% anhydrous methanol/HCl, and the methylated acids were further derivatised by *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Concentrations of individual compounds were evaluated using Hewlett-Packard 6890 gas chromatograph (GC) fitted with a HP7683 Series auto-sampler. A HP-1MS 60 m capillary column with 320 μm diameter and 0.25 μm film thickness was used with Helium as the carrier gas. The GC oven was temperature programmed as 40 °C for 1 min, 150 °C at the rate of 10 °C/min and then climbing to 315 °C at the rate of 6 °C/min with a final isothermal period of 20 min. GC peaks were identified by comparing with previous published spectra in which the peaks were determined using GC–MS (Logan and Eglinton, 1994; Logan et al., 1995; Huang et al., 1995, 1996; Lockheart et al., 2000).

2.5. Hydrogen isotope analyses

Gas chromatography/thermal conversion/isotope ratio mass spectrometry (GC/TC/IRMS) was performed to determine D/H compositions on individual lipid compounds (Wang and Huang, 2001; Pond et al., 2002). The temperature program and capillary column used were identical to those for GC analyses. GC separated compounds pass through an alumina tube which is heated to 1440 °C to convert organic H to H_2 (Burgoyne and Hayes, 1998), and the H_2 is then introduced into a Delta^{plus}XL isotope ratio mass spectrometer (Hilkert et al., 1999). Four isotope standards (5, α-androstane, *n*-C_{21} *n*-alkane, myristic acid methyl ester, and behenic acid methyl ester) were co-injected after every six injections of samples to monitor the analytical accuracy, and each sample was repeatedly measured in triplets.

Methylated fatty acids were further derivatized as trimethylsilyl ethers using BSTFA (Sessions et al., 1999). Correction for the derivatization was calculated by using the following formula (1) before reporting.

$$\delta D_{\text{real}} = \frac{(2n+2) \times \delta D_{\text{measured}} + 123.7 \times 3}{(2n-1)} \quad (1)$$

In formula (1), n refers to carbon number of the studied compound. To derive the formula, disodium salt of succinic acid (Aldrich) was measured for its δD value using thermal conversion/elemental analyzer/isotope ratio monitoring mass spectrometry (TC/EA/IRMS). The salt is then acidified with 1 N HCl and extracted with ethyl acetate. The succinic acid obtained is methylated using the same reagents and procedure as used for sample compounds. The isotopic difference before and after derivatization is used to calculate the δD value for the hydrogens on the methyl groups added (Huang et al., 1999). We obtained a value of -123.7 ± 2‰ (5 replicated measurements). Potential isotopic effect during derivatization was examined by heating the palmitic acid and tetrcosanoic acid (standards) with the same derivatization agents for different times (17, 24, 43 h) and at different temperatures (50, 80, 90 °C). We observed virtually no isotopic difference (within analytical error) between each derivatizations. Linearity of hydrogen isotope measurements has been tested using 9 pulses of standard hydrogen gas with an amplitude between 1V and 9V. After H_3 factor correction, the difference in δD values between peaks of different amplitudes is within ±2‰. δD values for peaks with amplitude <0.5 V are not reported. The analytical system has achieved standard deviations below 2‰ for all four isotope standards, and for the majority of examined ancient samples, the standard deviations were below 3‰. Poor precisions with standard deviations up to 11.8‰ were occasionally obtained for some compounds in which the peaks were either too small or co-elution occurred (e.g., C_{25}–C_{26} alkanes in *Salix*).

Water samples were analyzed using TC/EA/IRMS (Hilkert et al., 1999). Each sample was manually injected 6–7 times (0.3 μl for each injection). Three isotope standards, VSMOW ($\delta D = 0$), standard light Antarctic precipitation (SLAP, $\delta D = -428$‰), and Greenland ice sheet precipitation (GISP, $\delta D = -189.7$‰), were analyzed between every 10 water samples for calibration. A regression line was then established between measured and real δD values of the three isotope standards. We have carried out over 200 analyses of these calibrations

so far, and in all cases, we have obtained a perfect linear relationship ($R^2 = 1$) between measured and real δD values of the standards. The standard deviation for water δD value was $< 1.5‰$.

The reported sediment water δD is the mean value of the measurement of three water samples collected from the Horizon 2B. Because δD values of cellular water from modern plants are not available and sediment water may not represent ancient tissue water, we calculate "apparent H isotope fractionation" to account for the isotopic offsets between lipids and various water sources. For each compound between lipids and environmental water, we use formula (2) where l represents the studied lipid compound and w is environmental water.

$$\text{Apparent fractionation}_{l/w} = 1000 \times \left[\frac{(\delta D_l + 1000)}{(\delta Dw + 1000)} - 1\right] \quad (2)$$

For calculating apparent H isotopic fractionation between water and lipid compounds of living plant leaves, the mean values of Clarkia surface water δD were used for *Salix* and environmental water, whereas the average δD of surface water from Moscow, Idaho, was applied to calculate the offset between water and lipids from *Platanus* and *Quercus*.

3. Results

3.1. Distribution of individual fossil lipids

GC separations of individual fossil lipids from hydrocarbon, alkanol, and acid fractions yielded distinct lipid concentration profiles for each fossil genus and sediment. Fig. 1 shows selected lipid distribution and concentration of the three fractions from different fossils and sediments. Our results compare favorably with the following published profiles of previously studied lipid compounds from Clarkia sediments and fossils: all three fractions from sediments (Logan and Eglinton, 1994; Huang et al., 1995), *n*-alkanes from *Platanus* (Lockheart et al., 2000), *n*-alkanols and *n*-acids from *Platanus* (Huang et al., 1995), and *n*-acids from *Quercus* (Logan et al., 1995). For *Salix*, a bell shaped profile with odd/even domination peaking at C_{31} was obtained for the hydrocarbon fraction (Fig. 1a); whereas the *n*-acid series displays a bimodal distribution

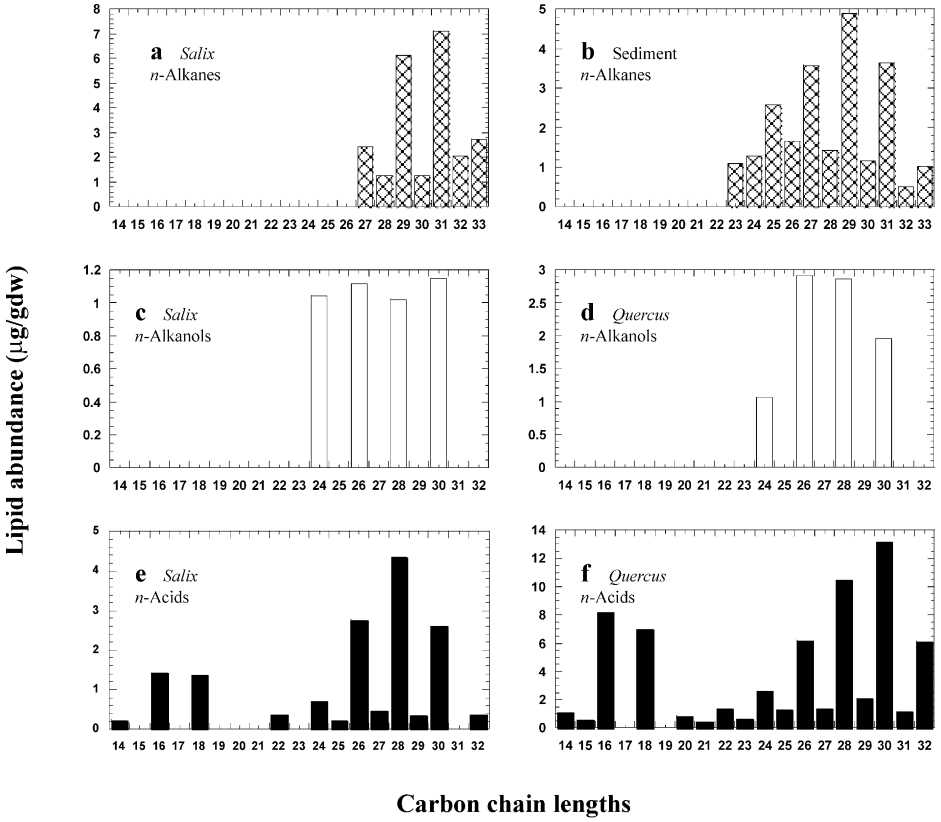

Fig. 1. Histograms showing the concentration and chain length distribution of *n*-alkanes, *n*-alkanols, and *n*-acids in selected leaf fossils and sediments from Clarkia.

with even/odd, maximizing at C_{28} (Fig. 1e). A homologous series of *n*-alkanols with strong even/odd predominance was observed (Fig. 1c). When different fossil genera appear to have similar profiles for the same compound class, different dominant peaks and concentrations distinguish them. For instance, the *n*-acids from fossil *Quercus* peak at C_{30} (Fig. 1f) whereas the dominant peak for *Salix* is at C_{28}.

3.2. Variation of δD in fossils and sediments

Table 1 displays measured individual-lipid *D*/*H* values for the two compound classes from both fossils and sediments. While the two sediment samples yielded similar or identical *δD* values for a particular compound (average difference of 0.7‰ for *n*-alkanes and 1.1‰ for *n*-acids), the D/H ratios vary among fossil genera and between fossils and sediments (except for *n*-C_{16} fatty acids where the fossils and sediments yielded almost identical *δD* values). For certain compounds, the offsets between *δD* values in fossils and sediments vary up to 60‰. In general, *δD* values of fossil *n*-alkanes are lower than those in sediments, whereas the pattern is reversed in *n*-acids. A range of *δD* values from −223 to −178‰ and −222 to −141‰ were obtained from fossil *n*-alkanes and *n*-acids respectively. The largest *δD* variation within the same compound class was obtained in *n*-acids (81‰) between *Platanus* C_{26} (−222‰) and *Salix* C_{21} (−141‰). *δD* variations within a particular compound vary among the two measured compound classes in Clarkia fossils with larger variations found in *n*-acids. *Salix* exhibits the largest *δD* variation among different compound classes with 83‰ difference between C_{21} *n*-acid (−141‰) and C_{31} *n*-alkane (−224‰). The average *δD* values of the hydrocarbons from the three fossils (−204, −206 and −209‰ for *Platanus*, *Quercus*, and *Salix* respectively) are slightly lower than that in fatty acids (−194, −203, and −191‰).

Several features are noticeable in Fig. 2, which graphically summarizes data from Tables 1 and 2. In both

Table 1
Hydrogen isotopic composition of individual compounds from Clarkia fossils and sediments

Carbon number	Sediment-1		Sediment-2		*Platanus*		*Quercus*		*Salix*	
	δD[a] (‰)	*σ*[b] (‰)	*δD* (‰)	*σ* (‰)	*δD* (‰)	*σ* (‰)	*δD* (‰)	*σ* (‰)	*δD* (‰)	*σ* (‰)
n-Alkanes										
23	−203	1.9	−201	1.7	−218	0.7	−211	1.7	N.D.[c]	N.D.
24	−195	2.4	−197	1.5	−204	4.7	−195	8.8	N.D.	N.D.
25	−204	1.8	−204	2.2	−214	0.4	−205	1.7	−195	10.5
26	−186	2.1	−181	3.0	N.D.	N.D.	N.D.	N.D.	−206	10.4
27	−209	0.5	−210	1.8	−212	3.1	−213	0.7	−207	0.4
28	−196	2.8	−194	2.1	−201	4.5	−194	2.5	N.D.	N.D.
29	−210	1.5	−213	0.7	−221	0.0	−216	0.6	−215	2.5
30	−182	2.4	−179	2.7	−195	10.1	−206	10.9	−208	4.8
31	−196	2.4	−196	1.4	−218	0.0	−214	3.3	−224	1.3
n-Acids										
16	−210	3.8	−212	0.3	−207	0.2	−212	1.1	−209	0.8
18	−196	1.3	−196	1.3	−145	0.7	−207	2.7	−186	2.4
20	−190	3.6	−194	0.7	−186	5.0	N.D	N.D.	−172	9.9
21	−203	11.7	−200	4.5	−178	2.1	N.D.	N.D.	−141	2.7
22	−207	3.1	−213	0.9	−192	7.6	−181	7.0	−181	4.4
23	−185	6.4	−188	1.3	−172	7.4	N.D.	N.D.	−150	5.1
24	−213	1.4	−215	0.3	−199	3.1	−206	3.4	−204	2.4
25	−193	3.0	−194	1.4	−182	1.3	−193	3.0	−179	2.7
26	−217	0.3	−217	0.6	−222	0.2	−221	0.5	−212	0.1
27	−199	1.5	−199	0.4	−194	1.3	−191	3.0	−189	3.4
28	−209	0.9	−209	0.5	−215	0.1	−218	0.1	−211	0.3
29	−203	1.9	−202	2.6	−208	0.5	−204	3.7	−204	0.7
30	−209	0.2	−209	0.5	−211	0.6	−214	0.1	−215	0.5
31	−199	2.4	−199	1.2	−201	3.3	−197	4.5	−212	5.0
32	−207	0.9	−207	0.9	−208	2.4	−204	1.3	−219	0.0

[a] *δD* values are expressed in ‰ relative to VSMOW.
[b] *σ* = standard derivation of three replicate measurements.
[c] N.D. = not determined.

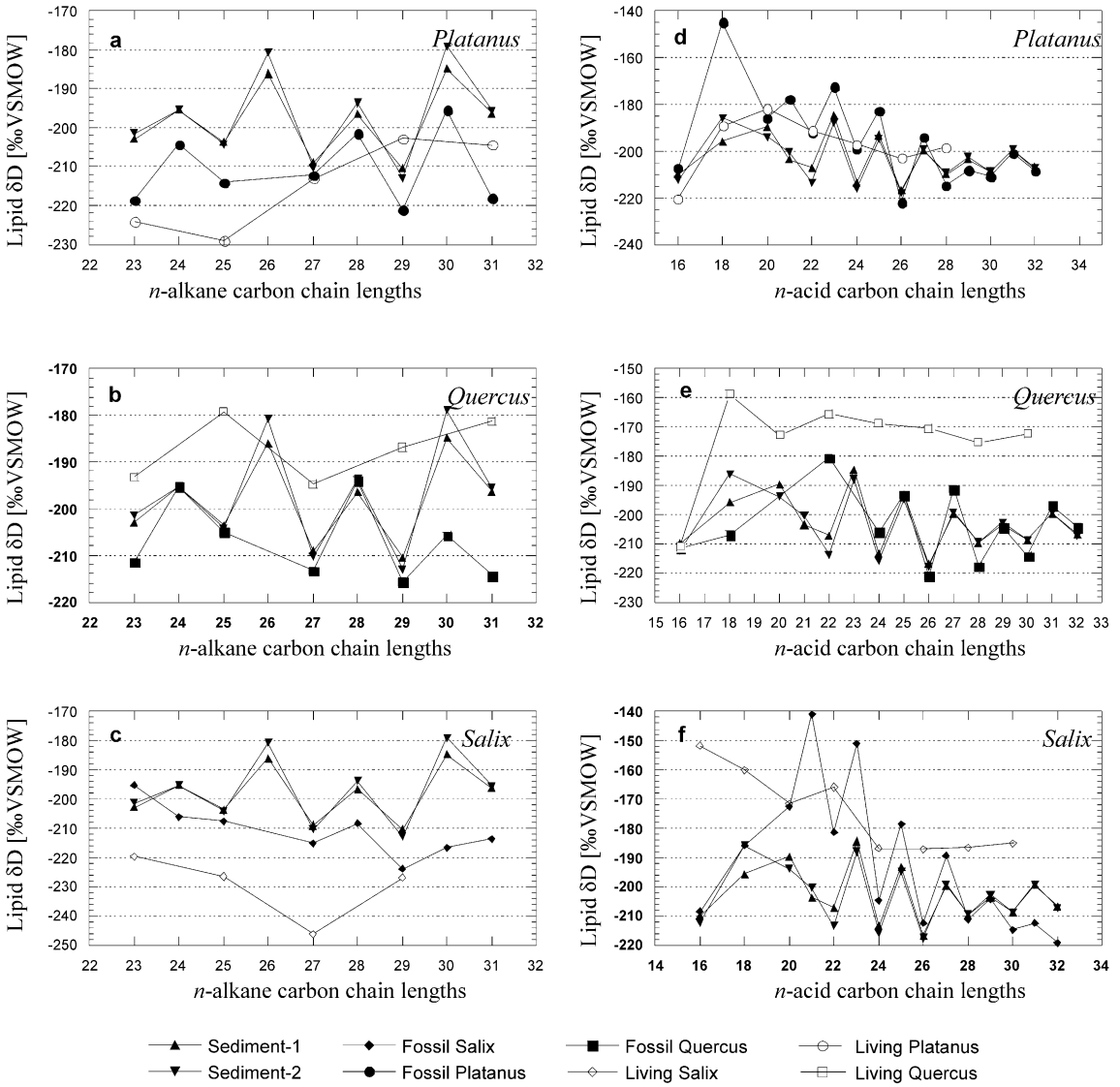

Fig. 2. Plots of δD values and chain length distribution of lipid from sediments, leaf fossils and leaves from living species: (a) *Platanus* *n*-alkanes, (b) *Quercus* *n*-alkanes, (c) *Salix* *n*-alkanes, (d) *Platanus* *n*-acids, (e) *Quercus* *n*-acids, and (f) *Salix* *n*-acids.

n-alkane and *n*-acid fractions, there is a regular δD offset pattern between even and odd C chains in both fossil and sediment samples. In *n*-alkanes, the odd carbon numbers are more enriched in D than they are in even chain carbons, whereas the pattern is reversed in *n*-acids with depleted D in even chain carbons. In both *Platanus* and *Salix* *n*-alkanes, the fossil lipid δD values are readily separated from those of the sediments by about 20‰ on average, whereas the values are similar in *Quercus*. For *n*-acid fractions of all three fossil genera, the δD values for *n*-C16 are similar or identical, and the δD value difference between fossil and sediment lipids tend to decrease with the increase of carbon chain length.

3.3. *Variation of* δD *in modern lipids*

Table 2 shows *n*-alkane and *n*-acid D/H compositions from lipids isolated from the three living species. A range of δD values between −246‰ to −179‰ (with a difference of 67‰), and −220 to −151‰ (with a difference of 69‰), were obtained for the hydrocarbon and acid fractions respectively. In all three species, fatty acids are more enriched in D contents relative to the hydrocarbons (56‰ on average). For a particular species, the δD variation in *n*-acids tends to be larger than that in *n*-alkanes, with *Quercus garryana* having the largest offset (62‰ in *n*-acids and 16‰ in *n*-alkanes

Table 2
Hydrogen isotopic composition of individual compounds from modern leaves collected from Idaho

Carbon number	*Quercus garryana*		*Platanus occidentalis*		*Salix amygdaloides*	
	δD (‰)	σ (‰)	δD (‰)	σ (‰)	δD (‰)	σ (‰)
n-Alkanes						
23	−224	1.8	−193	1.8	−220	1.1
25	−229	0.8	−179	0.6	−226	1.5
27	−213	1.8	−195	0.6	−246	1.3
29	−203	0.6	−187	0.8	−227	1.9
31	−204	0.1	−181	1.1	N.D.	N.D.
n-Acids						
14	−210	0.8	−198	0.8	−163	1.8
16	−220	2.0	−211	0.4	−151	1.1
18	−189	10.1	−159	2.5	−160	0.7
20	−182	4.4	−172	3.7	−171	11.3
22	−191	10.1	−165	2.0	−166	1.3
24	−197	4.3	−168	2.3	−187	1.3
26	−203	3.4	−170	1.6	−187	0.4
28	−198	11.2	−175	0.8	−186	0.6
30	N.D.	N.D.	−172	1.5	−185	2.4

respectively). Similar to fossils, *Salix amygdaloides* exhibits the largest δD variation among different compound classes (95‰, between C_{27} *n*-alkane and C_{16} *n*-acid). The variations of δD values within each species are rather small. For *n*-alkanes, a difference of 26‰ (between C_{23} and C_{29}), 16‰ (between C_{25} and C_{27}), and 26‰ (between C_{23} and C_{27}) was recorded for *Quercus*, *Platanus,* and *Salix* respectively; whereas δD variations in *n*-acid fractions are 38‰ (between C_{20} and C_{16}), 52‰ (between C_{16} and C_{18}), and 36‰ (between C_{16} and C_{26}) for *Quercus*, *Platanus,* and *Salix*. In addition, *n*-alkanes in living plants are more depleted in D contents than that in *n*-acids, a pattern that is consistent with what is observed in the fossils. On average, *n*-alkanes from modern leaves are more depleted in D than they are in their fossil counterparts (except for *Quercus*), whereas the pattern is reversed in *n*-acids in which fossil compounds generally show lower δD values. In most cases, the δD from living species is readily separated from either fossil or sediments. Comparing δD of the same compound in the living species, the fossil compounds may contain either depleted (e.g., in Fig. 2a and c) or enriched (e.g., in Fig. 2b and d) D contents.

3.4. Water δD and hydrogen isotope offsets

The observation that sediment waters obtained directly from sediment seeping and from immediate condensation within plastic bags yielded indistinguishable δD values (−113 and −114‰ respectively) indicates that no significant isotopic fractionation has occurred during our sampling process. Table 3 displays mean δD values from water samples collected from various sources in Clarkia and the surrounding areas and calculated apparent H isotope fractionations between lipids and these waters. The mean δD values vary slightly among water samples obtained from Clarkia sedimentary rocks, surface water collected near the P-33 site, and from Moscow, Idaho. The surface waters are slightly enriched in D in relation to the sediment water from the Clarkia rock formation. Compared with the sediment water, the fossil lipid δD values show hydrogen isotope offsets (apparent fractionation) ranging from 88 to 108‰, whereas the difference between environmental waters and *n*-alkane or *n*-acid fractions of modern plants displays a variation from 81 to 144‰ (Table 3).

Table 3
δD in surface and sediment waters, apparent hydrogen isotope fractionation between waters and lipids

	Clarkia surface	Clarkia sediment	Moscow surface
Hydrogen isotope in waters			
Mean δD ($n=3$)	−101±2.8‰	−114±1.3‰	−104±1.6‰
	Platanus	*Quercus*	*Salix*
Apparent hydrogen isotopic fractionation			
Fossil			
Alkanes/water	−103‰	−105‰	−108‰
Acids/water	−91‰	−102‰	−88‰
Living			
Alkanes/water	−124‰	−93‰	−144‰
Acids/water	−106‰	−82‰	−81‰

4. Discussion

4.1. Preservation of carbon-bound hydrogen isotope ratios in lipids

Our study represents the first attempt to measure compound-specific δD from identifiable Tertiary plant fossils. The organic rich lacustrine sediments at the Clarkia site provide a test for the preservation of primary D/H signatures in depositional environment associated with abundant clay minerals and saturated acidic water. Several lines of evidence led us to believe that the δD variations observed in these fossils and sediments suggest preservation of primary hydrogen isotopic compositions in the Clarkia deposit.

The large δD variations detected within each compound class between sediments and fossils (up to 62‰ in

n-C_{21} fatty acids) and among fossils (up to 62‰ between *Platanus* and *Quercus* in n-C_{18} acids) are indicative of the preservation of original hydrogen isotopic compositions in these fossils. If extensive diagenetic alternations had occurred, one would expect a more homogenous δD for compounds of similar structure and a small δD variation. As indicated in Fig 2, δD curves of both fractions from *Platanus* and *Salix* are readily separated from the sediment δD curve with the average difference about 20‰ (Fig. 2a, d, c and f). In *Quercus*, the δD offsets between fossil lipids and sediments are smaller (most within 15‰), but several compounds (e.g., n-C_{30} alkane, and n-C_{22} acid) yield large δD difference up to 27 and 32‰ (Fig. 2b and e). Extensive equilibration would tend to make co-occurring homologous alkyl lipids have similar δD values given the long time of depositional history for the Clarkia deposit. It is also evident, especially in short C chain n-acids, homologous lipids from fossil material exhibit large δD variations (e.g., n-C_{18} and n-C_{21}), although homologous series from fossils and sediments show similar trend of saw-tooth pattern (further discussions are given below). Such large variations in homologous n-alkyl lipids argue for small diagenetic alternation on the hydrogen isotope ratios of lipids.

The fossil δD variation pattern of inter-compound class falls within the range of lipid δD variations observed in modern higher plants. With a few exceptions (e.g. *Platanus* C_{18} n-acids), most of the δD variations within compounds are less than 30‰ for the fossil species, which is consistent with previous observation on living plants (Sessions et al., 1999) as well as additional data obtained from living plants in this study. In two higher plant species examined by Sessions et al. (1999), less than 50‰ δD variations were detected within n-alkanes. Compared with other two genera, substantial δD variations in individual lipids across different n-acids were observed in both living and fossil *Salix* (35‰ and 68‰, respectively, Fig.f). Since isotopic variability of individual lipids is largely controlled during biosynthesis processes (Martin et al., 1986), the similar behavior of δD variations found in the fossils and their living counterparts favor the interpretation of preservation of primary δD in Clarkia fossils.

It should be noted that although δD values are different between fossil and sediment samples, on average, the δD offsets between living and fossil leaves are greater than those between fossils and sedimentary groundmass. A synchronized saw-tooth lipid δD pattern between fossils and sediments was apparent in Fig. 2, and a similar pattern was also observed on compound specific C isotopes of hydrocarbons from the Clarkia site (Huang et al., 1995). Unfortunately, because the exceedingly low lipid concentration from odd C series in n-acids and even C series in n-alkanes from living plants prevented an accurate measurement of their δD values, whether these modern plants possess those similar isotopic characters is presently unclear. While the cause of this pattern in fossil material is largely unknown, there are several plausible explanations. This trend of similarity between fossils and sediments is expected as the majority of preserved n-alkyl lipids in the lake sediments were derived from the terrestrial plant community near the ancient Clarkia lake (Logan and Eglinton, 1994; Huang et al., 1995, 1996). In our dataset, especially in fatty acids, we have noticed that the δD differences between fossils and sediments tend to be larger in shorter C chain lipids (with the exception of C_{16}) than they are in longer C chain ($C > 26$). This general trend may be partially explained by the different origins of these lipids in fossils and sediments. Since the majority of long chain fatty acids in sediments derived from terrestrial higher plants, they tend to possess similar δD in comparison with lipids isolated from fossil materials, especially in long C chain n-alkanes. On the other hand, since algae produce large amounts of short chain fatty acids in sediments (Volkman et al., 1998), these short chain fatty acids are expected to have larger hydrogen isotope difference in δD values when compared with their higher plant fossil homologous. It is impossible to completely exclude sediments that are intimately associated with fossil tissues during sample preparation, thus minor sediment contamination is unavoidable. However, the distinct lipid profiles obtained from fossil and sediments as well as the large δD variations detected among fossil genera and between fossils and sediments indicate that such a problem is only minor.

4.2. Hydrogen isotope exchanges between water and buried organic molecules?

Hydrogen isotope exchange between organic molecules preserved in sedimentary rocks and the surrounding environment is a serious concern among geochemists who wish to apply compound-specific D/H analysis to ancient organic molecules in solving geological problems (Andersen et al., 2001; Sessions, 2001). Our results indicate that an extensive H exchange between environmental waters and fossil lipids is unlikely at the Clarkia site. The excellent molecular preservation and little diagentic alteration of δD values in Clarkia fossils and sediments imply minimal H isotope exchange between organic molecules and environmental water. The large hydrogen isotope offsets (up to 123‰) between fossil lipids and sediment water at the Clarkia site (Table 3) can be taken as evidence against extensive H exchange between water and fossil lipids. Although the age of the sediment water cannot be directly dated, it is possible that the sediment water represents ancient formation water. The excellent preservation of various organic molecules and compounds in the Clarkia fossils and sediments precludes oxygenated groundwater infiltration to these ancient lake sediments. Although the δD values in sediment water may not represent the original isotopic

values of the ancient lake waters, these waters have been associated with Clarkia sediments and fossils, thus allowed ample time for possible isotope exchange to proceed. Because the large mass difference between H in environmental water and in organic lipids in fossils, had an extensive H exchange occurred, more homogenous δD values between the sediment water and fossil lipids would be expected.

The complexity of a series of H isotope fractionation steps from environmental water to the point of lipid synthesis in terrestrial plants (Smith and Ziegler, 1990; Roden et al., 2000) made a straightforward comparison between D/H variation patterns of fossil and living species difficult. Available information from studies of cellulose and plant bulk organic matter have indicated that D/H ratios of organic compounds in terrestrial plant leaves are influenced by two major fractionations in opposite directions (Ziegler, 1989; Terwilliger and DeNiro, 1995). Evapotranspiration leads to an enrichment in D in the leaf waters (Epstein et al., 1977; Yakir, 1992; Terwilliger and DeNiro, 1995), whereas lipid biosynthesis processes involve fractionation against D (Smith and Epstein, 1970; Sternberg et al., 1984; Sternberg, 1988; Ziegler, 1989). Unlike aquatic plants which have lipid δD values directly related to the hydrogen isotope composition of the medium water (Sternberg, 1988; Sessions et al., 1999; Andersen et al., 2001), the influence of H isotopic composition due to evaporation has to be accounted for in terrestrial plants. Using environmental water to calculate hydrogen isotope fractionation in terrestrial plants results in a lowered fractionation value for lipid biosynthesis from leaf water. In addition, a daily fluctuation of H isotopes up to 20‰ in leaf water has been observed in living C_3 plants (Leaney et al., 1985), which suggests further caution to derive a generalized picture for H isotope fractionation during lipid biosynthesis in terrestrial plant leaves. These cascading fractionation events complicate our comparison of observed δD offset patterns between lipids and environmental waters (Table 3) in fossil and living species. In our case, the nature of sediment water associated with fossils and the leaf water from living plants were either unknown or not determined, and the environmental water we used for calculating the apparent H isotope fractionation was sampled during a short duration. We therefore cannot exclude the possibility that the similar pattern in apparent H isotope fractionation between living plants and fossil samples was merely fortuitous. Nonetheless, our primary interest is to assess the diagenetic effects on the H isotope ratios of sedimentary and fossil lipids. The large apparent H isotope fractionation values between surrounding water and lipids (Table 3) would suggest insignificant diagenetic alteration of H isotope values and imply minimal D/H exchange during burial of these fossil material for the past 15–20 million years.

There is a possibility that the growth water of Clarkia Miocene plants was much more D-depleted, and subsequent D/H exchange has enriched all preserved fossil compounds to produce a similar offset patterns between water and fossil compounds as is now seen between modern plants and environmental waters. However, this scenario is highly unlikely. The more D-depleted ancient growth water would suggest a colder paleoclimate in Clarkia during middle Miocene time when the weather was actually much warmer and more humid than that of present day based on paleobotanical evidence (Smiley and Rember, 1985; Yang, 1996).

Our observation is generally in agreement with a previous study on biogeochemistry of hydrogen isotopes in microalgae, which concluded that C bound H does not exchange with medium water (Estep and Hoering, 1980). While our data suggest no extensive D/H exchange has occurred in Clarkia samples, it should be noted, however, small amounts of H exchange were difficult to detect. Nevertheless, our results support a recent observation on δD values from sulfur-bound lipids in the 6 million year old sediments from the Mediterranean Sea, indicating that primary values of preserved D/H in individual compounds from ancient sediments can be used to reconstruct paleoenvironmental conditions (Andersen et al., 2001). The apparently faster exchange rates obtained through laboratory simulation (Sessions, 2001) may be attributed to results of experimental artifact.

4.3. *Variation of* δD *in modern plant lipids*

Sessions et al. (1999)'s report was the only publication with in-depth discussion on hydrogen isotope variations in individual compounds from living higher plants. Our analysis of the three living terrestrial plant species added new information on hydrogen isotope biochemistry in *n*-alkyl lipids from angiosperms. We observed that in all three living species examined, the hydrocarbons were more depleted in D relative to fatty acids (up to 56‰ in *Salix*). This is a reversed pattern from what has been observed on *Daucus carota* (carrot) by Sessions et al. (1999), but is consistent, both in the order and in the scale, with a previous report by Estep and Hoering (1980). A similar pattern with more negative hydrocarbon δD values than those in fatty acids is observed in fossil lipids, although the differences are considerable smaller (up to 18‰ in *Salix*). The lipids from Clarkia sediments, which reflect an average of organic input from surrounding higher plants, have a slightly different pattern than that from the fossils, with hydrocarbons enriched in D relative to fatty acids. No obvious trend toward enrichment in D with the increase of carbon number was observed from the modern species. Thus, more studies are needed before a clear pattern of isotope fractionation in higher plants can be fully established.

5. Conclusions

We have obtained distinct δD values from individual lipids of *n*-alkanes and *n*-acids isolated from three plant fossils (*Platanus, Quercus*, and *Salix*) as well as sediments from the Clarkia Miocene deposit in northern Idaho, USA. The large δD variations within and across compound classes between plant fossils and sediments as well as among the three fossil plant genera support an indigenous origin for these hydrogen isotopic values. These data are in agreement with previous observations of exceptional lipid preservation in the Clarkia plant material, and indicate that original hydrogen isotopic compositions are recognizable from Clarkia plant fossils and sediments after burial of 15 million years or more. The fidelity of fossil D/H ratios and the large δD offset between fossil lipids and sediment water suggest that diagenetic alteration of original hydrogen isotope ratios is most likely only small at the Clarkia site. The reproducible results further warrant the feasibility of applying lipid-bound hydrogen isotopes to paleoecological and paleoenvironmental studies in Tertiary deposits.

Acknowledgements

We thank Edward Golenberg for additional samples, Yi Wang and Valerie E. Chamberlain for technical and field assistance, Peg Smiley and Kourtney Kienbaum for water samples, Richard Yund for microscopic examination of samples, and Emily and Crystal Yang for initial sample processing. Francis Kienbaum is acknowledged for permission to collect fossil material and water samples from his Clarkia property. Edward Golenberg, Alex Sessions, Megan Fuller, and Kristy Pond read the manuscript and provided constructive comments. We also thank Nils Andersen and two anonymous referees for criticism and suggestions. The project was financially supported by an American Chemical Society Petroleum Research Fund (ACS-PRF# 35208-G2) and a US NSF grant (ATM-0081478) to Y. Huang, and an American Chemical Society Summer Research Fund, the National Science Foundation of China (NSFC-40028201), and Bryant College research stipend to H. Yang.

Associate Editor—**G. Abbott**

References

Andersen, N., Paul, H.A., Bernasconi, S.M., McKenzie, J.A., Behrens, A., Schaeffer, P., Albrecht, P., 2001. Large and rapid climate variability during the Messinian salinity crisis: evidence from deuterium concentrations of individual biomarkers. Geology 29, 799–802.

Burgoyne, T.W., Hayes, J.M., 1998. Quantitative production of H_2 by pyrolysis of gas chromatographic effluents. Analytical Chemistry 70, 5136–5141.

Epstein, S., Thompson, P., Yapp, C.J., 1977. Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198, 1209–1215.

Estep, M.F., Hoering, T.C., 1980. Biochemistry of the stable hydrogen isotopes. Geochimica et Cosmochimica Acta 44, 1197–1206.

Giannasi, D.E., Niklas, K., 1981. Comparative paleobiochemistry of some fossil and extant Fagaceae. American Journal of Botany 68, 762–770.

Golenberg, E.M., Giannasi, D.E., Clegg, M.T., Smiley, C.J., Durbin, M., Henderson, D., Zurawski, G., 1990. Chloroplast DNA sequence from a Miocene *Magnolia* species. Nature 334, 656–658.

Hilkert, A.W., Douthitt, C.B., Schluter, H.J., Brand, W.A., 1999. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. Rapid Communication in Mass Spectrometry 13, 1226–1230.

Huang, Y., Lockheart, M.J., Collister, J.W., Eglinton, G., 1995. Molecular and isotopic biogeochemistry of the Miocene Clarkia Formation: hydrocarbons and alcohols. Organic Geochemistry 23, 785–801.

Huang, Y., Lockheart, M.J., Logan, G.A., Eglinton, G., 1996. Isotope and molecular evidence for the diverse origins of carboxylic acids in leaf fossils and sediments from the Miocene Lake Clarkia deposit, Idaho, USA. Organic Geochemistry 24, 289–299.

Huang, Y., Street-Perrott, F.A., Perrott, F.A., Metzger, P., Eglinton, G., 1999. Glacial–interglacial environmental changes inferred from the molecular and compound- specific $\delta^{13}C$ analyses of sediments from Sacred Lake, Mt Kenya. Geochimica et Cosmochimica Acta 63, 1383–1404.

Hunkeler, D., Andersen, N., Aravena, R., Bernasconi, S.M., Butler, B.J., 2001. Hydrogen and carbon isotope fractionation during aerobic biodegradation of benzene. Environmental Science and Technology 35, 3462–3467.

Leaney, F.W., Osmond, C.B., Allison, G.B., Ziegler, H., 1985. Hydrogen-isotope composition of leaf water in C_3 and C_4 plants: its relationship to the hydrogen-isotope composition of dry matter. Planta 164, 215–220.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P., 2000. Chemotaxonomic classification of fossil leaves from the Miocene Clarkia lake deposit, Idaho, USA based on n-alkyl lipid distributions and principal component analyses. Organic Geochemistry 31, 1223–1246.

Logan, G.A., Boon, J., Eglinton, G., 1993. Structural biopolymer preservation in Miocene leaf fossils from the Clarkia site, northern Idaho. Proceedings of the National Academy of Sciences USA 90, 2246–2250.

Logan, G.A., Eglinton, G., 1994. Biochemistry of the Miocene lacustrine deposit, at Clarkia, northern Idaho, USA. Organic Geochemistry 21, 857–870.

Logan, G.A., Smiley, C.J., Eglinton, G., 1995. Preservation of fossil leaf waxes in association with their source tissues, Clarkia, northern Idaho, USA. Geochimica et Cosmochimica Acta 59, 751–763.

Martin, G.J., Zhang, B.L., Naulet, N., Martin, M.L., 1986. Deuterium transfer in the bioconversion of glucose to ethanol

studied by specific isotope labeling at the natural abundance level. Journal of the American Chemical Society 108, 5116–5122.

Niklas, K.J., Giannasi, D.E., Baghai, N.L., 1985. Paleobiochemistry of a North American fossil *Liriodendron* sp. Biochemical Systematics and Ecology 13, 1–4.

Pond, K.L., Huang, Y., Wang, Y., Kulpa, C.F., 2002. Hydrogen isotopic composition of individual *n*-alkanes as an intrinsic tracer for bioremediation and source identification of petroleum contamination. Environmental Science and Technology 36, 724–728.

Rieseberg, L.H., Soltis, D.E., 1987. Flavonoids of fossil Miocene *Platanus* and its extant relatives. Biochemical Systematics and Ecology 15, 109–112.

Roden, J.S., Lin, G., Ehleringer, J.R., 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. Geochimica et Cosmochimica Acta 64, 21–35.

Sauer, P., Eglinton, T.I., Hayes, J.M., Schimmelmann, A., Sessions, A., 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. Geochimica et Cosmochimica Acta 65, 213–222.

Sessions, A.L., 2001. Hydrogen Isotope Ratios of Individual Organic Compounds. PhD dissertation, Indiana University, Bloomington, Indiana, USA, 149p.

Sessions, A.L., Burgoyne, T.W., Schimmelmann, A., Hayes, J.M., 1999. Fractionation of hydrogen isotopes in lipid biosynthesis. Organic Geochemistry 30, 1193–1200.

Smith, B.N., Epstein, S., 1970. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. Plant Physiology 46, 738–742.

Smith, B.N., Ziegler, H., 1990. Isotopic fractionation of hydrogen in plants. Botanica Acta 103, 335–342.

Smiley, C.J., Gray, J., Huggins, L.M., 1975. Preservation of Miocene fossils in unoxidized lake deposits, Clarkia, Idaho. Journal of Paleontology 49, 833–844.

Smiley, C.J., Rember, W.C., 1985. Physical setting of the Miocene Clarkia fossil beds, northern Idaho. In: Smiley, C.J. (Ed.), Late Cenozoic History of the Pacific Northwest. Pacific Division of American Association for the Advancement of Science, San Francisco, CA, pp. 11–30.

Soltis, P.S., Soltis, D.E., Smiley, C.J., 1992. An *rbc*L sequence from a Miocene *Taxodium* (bold cypress). Proceedings of the National Academy of Sciences USA 89, 449–451.

Sternberg, L.S.L., 1988. D/H ratios of environmental water recorded by D/H ratios of plant lipids. Nature 333, 59–61.

Sternberg, L., DeNiro, M.J., Ajie, H., 1984. Stable hydrogen isotope ratios of saponifiable lipids cellulose nitrate from CAM, C_3 and C_4 plants. Phytochemistry 11, 2475–2477.

Terwilliger, V.J., DeNiro, M.J., 1995. Hydrogen isotope fractionation in wood-producing avocado seedlings: biological constraints to paleoclimatic interpretations of δD values in tree ring cellulose nitrate. Geochimica et Cosmochimica Acta 59, 5199–5207.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. Organic Geochemistry 29, 1163–1179.

Wang, Y., Huang, Y., 2001. Hydrogen isotope fractionation of low molecular weight *n*-alkanes during progessive vaporization. Organic Geochemistry 32, 991–998.

Ward, J.A.M., Ahad, J.M.E., Lacrampe-Couloume, G., Slater, G.F., Edwards, E.A., Lollar, B.S., 2000. Hydrogen isotope fractionation during methanogenic degradation of toluene: potential for direct verification of bioremediation. Environmental Science and Technology 34, 4577–4581.

Xie, X., Nott, C.J., Avsejs, L.A., Volders, F., Maddy, D., Chambers, F.M., Gledhill, A., Carter, J.F., Evershed, R.P., 2000. Paleoclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. Organic Geochemistry 31, 1053–1057.

Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant Cell and Environment 15, 1005–1020.

Yang, H., 1993. Miocene Lake Basin Analysis and Comparative Taphonomy: Clarkia (Idaho, U.S.A.) and Shanwang (Shandong, P.R. China). PhD dissertation, University of Idaho, Moscow, 272p.

Yang, H., 1996. Comparison of Miocene fossil floras in lacustrine deposits: implications for palaeoclimatic interpretations at the middle latitude of the Pacific rim. Palaeobotanist 45, 416–429.

Yang, H., Smiley, C.J., Sprenke, K.F., Rember, W.C., Knowles, C.R., 1995. Subsurface evidence for a rapid formation of the Clarkia Miocene lake in northern Idaho. Northwest Science 69, 52–59.

Ziegler, H., 1989. Hydrogen isotope fractionation in plant tissues. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), Stable Isotopes in Ecological Research. Springer-Verlag, New York, pp. 105–123.