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Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions

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Abstract—Hydrogen isotope ratios (D/H) of lipid biomarkers extracted from aquatic sediments were measured to determine whether they can be used as a proxy for D/H of environmental water. Values of δD were determined by using a recently developed isotope-ratio-monitoring gas chromatograph-mass spectrometer system (irmGCMS) and were confirmed by conventional hydrogen isotopic measurements (i.e., combustion followed by reduction) on individual compounds isolated by preparative capillary gas chromatography. Diverse lipids (alkanes, *n*-alkanols, sterols, and pentacyclic triterpenols) were analyzed to examine hydrogen-isotopic controls on lipids of varying origin and biosynthetic pathway. For algal sterols (24-methylcholest-3β-ol, 24-ethylcholest-5,22-dien-3β-ol, and 4,23,24-trimethylcholesterol, or dinosterol), the fractionation between sedimentary lipids and environmental water was -201 ± 10‰ and was similar in both marine and freshwater sites. In a sediment from a small lake in a forested catchment, triterpenols from terrestrial sources were enriched in D by 30‰ relative to algal sterols. Apparent fractionation factors for *n*-alkyl lipids were smaller than those of triterpenols and were more variable, probably reflecting multiple sources for these compounds. We conclude that hydrogen-isotopic analyses of algal sterols provide a viable means of reconstructing D/H of environmental waters. Results are less ambiguous than reconstructions based on analyses of kerogen or other operationally defined organic matter fractions. *Copyright © 2001 Elsevier Science Ltd*

1. INTRODUCTION

Isotope ratios of hydrogen (D/H) and oxygen ($^{18}O/^{16}O$) in precipitation are strongly influenced by environmental variables (Dansgaard, 1964; Rozanski et al., 1993), making isotopic proxies of past precipitation valuable in paleoclimate studies. The past isotopic composition of precipitation has been estimated from isotope ratios in ice cores (e.g., Dansgaard et al., 1973; Johnsen et al., 1995), speleothems (e.g., Winograd et al., 1997), tree rings (e.g., Gray and Thompson, 1976; Epstein and Yapp, 1976; Gray and Song, 1984; Epstein and Xu, 1999; Tang et al., 2000), lacustrine sediment using cellulose (e.g., Edwards and McAndrews, 1989; Wolfe et al., 1996) or carbonate (e.g., Kelts and Talbot, 1990), and authigenic clay minerals (Elliott et al., 1997; Savin and Hsieh, 1998; Chamberlain et al., 1999). Because the physical processes controlling fractionation of oxygen and hydrogen isotopes within the hydrologic cycle are the same, $\delta^{18}O$ and δD are strongly correlated in precipitation and, to a first approximation, their signals can be used interchangeably (Dansgaard, 1964). However, $\delta^{18}O$ and δD are not perfectly correlated because of differences in fractionation that can yield information about nonequilibrium processes in the hydrologic cycle such as evaporation (Dansgaard, 1964; Gonfiantini, 1986).

Lake sediments are particularly useful archives of paleoclimate information because they can provide continuous time series and contain multiple geochemical and microfossil proxies which can be used to verify interpreted paleoclimate signals (Birks and Birks, 1980; Faegri et al., 1989; Smol, 1995; Meyers and Lallier–Vergès, 1999). However, lake-sediment based paleoisotopic studies are frequently hampered by difficulties in isolating a purely autochthonous sedimentary material. For example, carbonate is not preserved in lakes with low pH, and kerogen (solvent-insoluble organic matter) or sedimentary cellulose can contain large proportions of terrigenous matter (Hedges and Mann, 1979; Hedges et al., 1997; Sauer et al., in press).

Lipid biomarkers are biochemicals that derive from a restricted range of organisms and thus provide a highly selective means of isolating material of specific origin. Examples include long-chain *n*-alkyl lipids (*n*-alkanes and *n*-alkanols) produced mainly by terrestrial plants (Fengel and Wegener, 1984) and certain sterols (e.g., 4-methyl sterols and others with specific patterns of alkylation or unsaturation) produced predominantly by phytoplankton (Volkman, 1986; Volkman et al., 1998). Other prominent components of lacustrine sedimentary lipids such as C_{16} and C_{18} fatty acids probably represent mixtures from terrigenous and aquatic sources. Most hydrogen in lipidic biomarkers is bound to carbon and is nonexchangeable, whereas kerogen, cellulose, and phenolic substances contain some exchangeable hydrogen (Schimmelmann et al., 1999).

To assess whether D/H ratios in autochthonous lipid biomarkers can be used to reconstruct isotopic compositions of paleolakewaters, we have analyzed algal sterols extracted from sediments of five lakes and two marine locations. Lakes were chosen to span a climatic gradient and were of varying size, depth, and productivity. Marine sites were chosen to represent different conditions of preservation.

We analyzed sedimentary lipids to establish the relationship

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Table 1. Locations, dates, and depths of samples analyzed in this study.

Location		Date sampled	Elevation (m above sea level)	Water depth (m)	Sediment depth (cm)
	Marine sites				
Mud Patch (Massachusetts, USA)	40°30′N, 70°45′N	April 1993	n/a	75	2–5
Santa Monica Basin (California, USA)	33°44.0′N, 118°50.0′W	Nov. 1996	n/a	905	0.75–1.5
	Lacustrine sites				
Avalaqiak Lake (Baffin Island, Canada)	66°47.9′N, 63°57.7′W	May 1998	14	12	0–2
Brother-of-Fog Lake (Baffin Island, Canada)	67°11.5′N, 63°8.0′W	May 1998	350	16	0–2
Duck Pond (Truro, Massachusetts, USA)	41°56.0′N, 70°00.1′W	June 1997	3	6	0–2
Lac Hertel (Quebec, Canada)	45°32.5′N, 73°09.1′W	July 1998	7	7	0–2
Snow Pond (Truro, Massachusetts, USA)	41°58.1′N, 70°01.9′W	June 1997	3	7	0–2
Great Pond (Truro, Massachusetts, USA)	41°58.5′N, 70°01.8′W	July 1998	3	9	0–2

between environmental controls and materials preserved in the geologic record. This provides a more direct and inclusive test of processes affecting isotopic records than would any examination of individual, living organisms.

2. MATERIAL AND METHODS

2.1. Study Sites and Field Sampling

Table 1 lists the names and geographic locations of study sites. Lake sediments were collected using a hand-held Ekman dredge working from small rafts or through holes drilled in ice. The top 2 cm was collected for analysis, roughly the depth of bioturbation based on the occurrence of midge larvae in productive lakes such as Avalaqiak Lake (arctic Canada). Water samples for isotopic analysis were collected either at a depth of 30 cm (summer) or from the clear water above sediments in the Ekman dredge samples. In Great Pond (Cape Cod, MA, USA), particulate organic matter (POM) was collected in July 1998 by filtering 4 L of water taken at 20 cm water depth through a precombusted Whatman glass fiber filter (GF/A) with a nominal pore size of 1.6 μm.

Water samples were collected at 30 cm depth in Spring (May) and summer (August) from Lac Hertel and Great Pond to measure seasonal differences in the isotopic composition of lakewater. The range of δD values was 4 and 2‰, respectively. Because of the remoteness of arctic field sites, it was not possible to visit these sites at different times of the year. However, by comparison to better-studied lakes with similar morphometric and watershed characteristics on Baffin Island (Sauer, 1997), we expect seasonal δD ranges of 10 to 20‰ for the arctic lakes in this study (Avalaqiak and Brother-of-Fog lakes) where spring runoff dominates annual inputs, but where evaporation during summer causes significant D enrichment. Smaller ranges of δD are expected in lakes in southern Canada and coastal Massachusetts, where there are smaller seasonal ranges in the amount and isotopic composition of precipitation, and groundwater (typically representative of annual average precipitation) forms a greater portion of total inflows.

Marine sediment samples were collected by Mark III box core (Mud Patch) or Ocean Instruments multicorer (Santa Monica Basin; Pearson, 1999). Equivalent depth intervals from several subcores were combined to increase sample size. The Mud Patch underlies well-oxygenated water at a depth of 75 m, whereas the Santa Monica Basin sample represents a basin with suboxic bottom water and anoxic sediment at 905 m water depth (Christensen et al., 1994). Gong and Hollander (1997) have shown that preservation of organic carbon at the anoxic depocenter of the Santa Monica Basin is 32% higher than that at an oxic site that is only 15 km distant and that receives the same inputs from the water column. Moreover, the character of the organic matter at the two sites differs significantly, with the atomic H/C ratio in organic matter at the depocenter being approximately 25% higher than that in the oxic sediments.

Samples of terrestrial vegetation and soils were collected to identify terrestrial sources of lipid biomarkers. Soil samples and leaves of dominant vegetation types were collected at Great Pond and Avalaqiak Lake (Table 1). Great Pond occupies forested watershed of scrub oak (*Quercus* spp.) and pitch pine (*Pinus rigida*) on a sandy soil that has not been cultivated in the 20th century. A 30-cm soil profile at Great Pond was analyzed for lipid biomarker concentrations. These samples represent varying degrees of degradation of all dominant sources of terrigenous organic material and thereby identify which biomarkers may represent terrestrial inputs.

2.2. Lipid Purification and Preparation

Sediments were air-dried at 40°C. For two sites (Great Pond and Lac Hertel), the total lipid extract (TLE) was obtained by Soxhlet extraction (dichloromethane/methanol 93/7, v/v, for 72–96 h) of 100 to 150 g sediment (dry weight). For the remaining samples, a TLE was obtained using accelerated solvent extraction (ASE, Dionex Corp. Schantz et al., 1997; Richter, 1999) with dichloromethane/methanol (9/1), 100°C, 1000 psi for 15 min. Lipids were separated by column chromatography (100–200 mesh silica gel) by using the elution sequence of Lee et al. (1977): F1 (hydrocarbons): hexane (100%), F2 (ketones, fatty acid methyl esters): ethyl acetate/hexane (15/85), F3 (alcohols): ethyl acetate/hexane (20/80), F4 (diols): ethyl acetate/hexane (25/75), F5 (fatty acids): methanol (100). Alcohols (F3) were separated into *n*-alkanols and polyisoprenoid alcohols by using urea adduction. Both alcohol fractions were acetylated by using acetic anhydride and pyridine (1/1), under N_2, and heating to 70°C (8 h). If samples contained 4-methyl sterols such as dinosterol, the acetylated 4-methyl and 4-desmethyl sterols were separated by silica-gel thin layer chromatography (TLC) by using diethyl ether/hexane (22/78) as the eluents. Stenols and stanols were separated using argentation TLC (10% $AgNO_3$ on 200-mesh silica gel) using hexane/dichloromethane (3/1 or 1/1, Morris, 1966).

To investigate the steryl component of phorbin steryl esters, the polar lipid fractions (F5) from several samples were transesterified in methanol/conc. hydrochloric acid (95/5) at 70°C for 12 h. Sterols were isolated from the resulting transesterification products by column chromatography as described above.

Concentrations of individual compounds were determined by using an HP5890 gas chromatograph (GC) equipped with a flow splitter that allowed simultaneous analyses on two different columns. The use of two columns provided a check for co-elution problems (compounds with identical retention times on one column often have different retention times on a column with a different stationary phase). The columns used were a Restek R_{tx}-200 column and either a J&W DB-1 or a J&W DB-5 column, all 60 m $\times$ 0.32 mm internal diameter (i.d.), 0.25 μm film thickness. Compounds were identified from mass spectra and gas chromatographic retention characteristics (HP6890 GC and HP5973 mass selective detector).

The isoprenoid alcohols from Great Pond were separated by preparative capillary gas chromatography (PCGC) by using an HP5890 GC fitted with a Gerstel preparative fraction collector as described by Eglinton et al. (1996). Both free-lipid and transesterified fractions were separated in this way. This allowed isolation of stenol/stanol pairs and pentacyclic triterpenols in sufficient quantity for conventional hydrogen isotopic analysis (i.e., sealed-ampoule combustion to water fol-

Table 2. Names and abbreviations of sterols in this study.

Systematic name	Trivial name	Abbreviation
androstan-3β-ol	androstanol	$19\Delta^0$
cholest-5-en-3β-ol	cholesterol	$27\Delta^5$
cholestan-3β-ol	cholestanol	$27\Delta^0$
24-methylcholest-5-en-3β-ol	campesterol	$28\Delta^5$
24-methylcholestan-3β-ol	campestanol	$28\Delta^0$
24-ethylcholest-5,22-dien-3β-ol	stigmastenol	$29\Delta^{5,22}$
24-ethylcholest-22-en-3β-ol	stigmastenol	$29\Delta^{22}$
24-ethylcholest-5-en-3β-ol	β-sitosterol	$29\Delta^5$
24-ethylcholestan-3β-ol	β-sitostanol	$29\Delta^0$
4,23,24-trimethylcholest-22-en-3β-ol	dinosterol	$d\Delta^{22}$
4,23,24-trimethylcholestan-3β-ol	dinostanol	$d\Delta^0$

lowed by reduction to H_2 using uranium turnings, Schimmelmann and DeNiro, 1993). Typically, 600 to 1200 μg of each compound were isolated for these analyses. Stenol-stanol pairs were incompletely resolved by this procedure. Accordingly, conventional isotopic analyses in some instances represent stenol + stanol mixtures. Throughout this paper, sterols are abbreviated as indicated in Table 2.

2.3. Isotope Ratio Monitoring Mass Spectrometry for D/H Determination

Samples were analyzed for D/H composition by using a recently developed system for isotope-ratio-monitoring gas chromatography-mass spectrometry (irmGCMS; Burgoyne and Hayes, 1998; Sessions et al., 1999). A Varian gas chromatograph was fitted with a Restek R_{tx}-200 or a J&W DB-5MS capillary column (both 30 m $\times$ 0.25 mm I.D. $\times$ 1 μm film thickness). The DB-5MS column provided superior chromatographic separation of hydrocarbons. We analyzed the larger, more polar compounds (i.e., steryl acetates) using the R_{tx}-200 column because it provided adequate chromatographic resolution with significantly shorter retention times. The column effluent passes through an alumina tube which is heated to 1400°C to quantitatively convert organic H to H_2 (Burgoyne and Hayes, 1998). The H_2 is then introduced into a Finnigan MAT 252 isotope ratio mass spectrometer. The mass spectrometer has been fitted with an electrostatic lens to separate scattered ^{4}He from the mass-3 ion beam. After correcting for the contribution of H_3^+ to the mass-3 beam (Sessions et al., 1999), it is possible to calculate the D/H ratios of individual compounds by integrating the mass-2 and mass-3 signals.

With our instrument, the most consistent results were obtained with peak heights of 250 to 5000 mV, measured at the mass-2 collector (amounts injected = 20–100 nmoles H_2 per compound on-column; 150–700 ng of each compound). The H_3^+ factor was determined by observing changes in the (mass-3)/(mass-2) ion-current ratio as the pressure of H_2 in the ion source was varied by adjustment of the variable-volume inlet. Values of δ were calculated relative to pulses of H_2 gas supplied from the variable-volume inlet and were calibrated against the VSMOW scale by reference to H_2 produced from co-injected primary standards (*n*-alkanes and fatty acid methyl esters available from A. Schimmelmann, Biogeochemical Laboratories, Indiana University). Reported values of δD represent averages of three to six replicate analyses. All δD values are reported in ‰ according to Coplen's (1996) guidelines relative to VSMOW (0‰) and normalized to VSLAP (−428‰).

2.4. Derivative H

To remove readily exchangeable hydroxyl hydrogen, we derivatized alcohols by using acetic anhydride to form the acetate ester. All acetylation was performed using the same batch of acetic anhydride, for which the δD was measured using conventional isotopic techniques (Schimmelmann, 1991).

To test whether any isotopic fractionation is associated with acetylation, we analyzed three sterol standards ($27\Delta^5$, $27\Delta^0$, and $19\Delta^0$) after derivatizing in various ways. The sterol standards were obtained from Sigma Scientific Co. (St. Louis, MO, USA) and were all >95% purity. Each sterol was acetylated by using acetic anhydride with known δD (−106‰) and by reaction with trifluoroacetic anhydride. The latter reagent contributes no hydrogen to the derivative. The $19\Delta^0$ and $27\Delta^0$ standards were also converted to stanones (ketones) using tetra-propylammonium perruthenate (TPAP, Griffith et al., 1987). D/H ratios were determined by conventional D/H analysis.

3. RESULTS AND DISCUSSION

3.1. Estimates of Uncertainty

The precision of D/H analyses of lipids is influenced by a number of factors, including those associated with mass spectrometric measurements of isotope ratios and, in compounds containing carboxyl or hydroxyl H, with effects related to derivatization. Errors specific to the irmGCMS measurements can also result from variations in the H_3^+ factor and from chromatographic problems such as closely eluting compounds. Because some compounds (e.g., *n*-alkanes) can be separated more easily than others (e.g., sterols), analytical uncertainties must be evaluated separately for each compound class.

Analytical uncertainties were estimated from results of replicate analyses. In earlier work, Sessions et al. (1999) found that the root-mean-square (RMS) error for hydrogen-isotopic analyses of well resolved *n*-alkanes for this instrument was 5.3‰. This result was based on 33 replicate analyses of a mixture of *n*-alkanes of known isotopic composition. Because it represents the RMS difference between the analytical result and the known isotopic composition, it is a measure of both accuracy and precision. For sterols, where co-elution poses a more significant problem, uncertainties are larger. Based on 38 injections of seven different mixtures of sterols (117 isotopic analyses of 32 sterols), the pooled estimate of the standard deviation of single observations is 8‰. For *n*-alkanols, the same procedure (9 injections, two different mixtures, 91 analyses of 25 different compounds) yields a pooled estimate of 7‰ for the standard deviation of a single observation. The uncertainty—expressed as a standard error—decreases with $1/\sqrt{n}$ when multiple measurements are made. In this paper, uncertainties are reported as 95% confidence limits based on the standard error for the appropriate compound class.

3.2. Analytical Tests

3.2.1. Derivatization

To measure isotopic fractionations associated with acetylation of alcohols, it is necessary to compare the δD of the acetylated alcohol to those of the derivatizing reagent (acetic anhydride) and of the C-bound H in the underivatized alcohol. The first two quantities can easily be determined by conventional isotopic analysis, but determination of the δD of C-bound H in hydroxyl-containing compounds is difficult because hydroxyl hydrogen exchanges readily with environmental moisture, often with poorly constrained isotopic shifts (Schimmelmann, 1991). Rather than using the laborious approach of exchanging hydroxyl H with water of known isotopic composition (Schimmelmann, 1991), we measured δD of C-bound H by using two techniques: oxidation of the sterol to the corresponding sterone, and derivatization with trifluoroacetic anhydride, which contains no H.

Values of δD for the variously derivatized sterol standards are reported in Table 3. The δD values reported for the acetates

Table 3. Hydrogen isotopic compositions of derivatives of sterols.

	δD_{VSMOW}, ‰[a]			
		C-bound H in parent sterol		
Compound	Steryl acetate	Trifluoroacetate	Stanone	Calculated from steryl acetate
$19\Delta^0$	−244	−248	−257	−257
$27\Delta^0$	−247	−253	−250	−256
$27\Delta^5$	−227	−231	n/a	−235

[a] Measured in duplicate by conventional (off-line) analysis. Based on replicate analysis of laboratory standards using this technique, 95% confidence interval = ±4‰.

include contributions from the C-bound H in the parent sterol and from the three H atoms in the acetate-methyl group. The δD values for the trifluoroacetyl esters include only the C-bound H in the parent sterol. Those of the stanones include the same H, less one atom lost during oxidation of the alcohol to a ketone. The yields of the trifluoroacetates were essentially quantitative. Accordingly, the steroidal H cannot have been isotopically fractionated during the derivatization. Apart from the effects of any involatile, H-bearing impurities contributed by the trifluoracetic anhydride, values of δD for the trifluoroacetates should accurately reflect the isotopic composition of the steroidal H. Yields of the stanones were lower. Even so, none of the H in the stanones could have been fractionated by a primary isotope effect. Secondary deuterium isotope effects (i.e., effects associated with substitution of D for H at carbon atoms adjacent to the site of oxidation), however, have the potential to cause some fractionation. On balance, isotopic analyses of the trifluoroacetates and of the stanones provide imperfect, but fully independent, estimates of the isotopic compositions of the C-bound H in the parent sterols.

The results summarized in Table 3 are mutually supportive. First, the average difference between the isotopic compositions of the trifluoroacetates and stanones is only 3‰. Either the C-bound H has not been fractionated by derivatization or the two different reactions happen to cause the same fractionation. Second, when the isotopic compositions of the C-bound H are calculated from those of the steryl acetates, assuming that the acetate H is unfractionated (δD = −106‰), similar results are obtained: $(\delta_{19\Delta0} \approx \delta_{27\Delta0}) \approx \delta_{27\Delta5} - 20‰$. Together, these results indicate that any of these approaches yields values of δD for the nonexchangeable H that are both precise (i.e., as a measure of differences between sterols) and, within limits that are discussed below, accurate (i.e., as a measure of the isotopic composition of the sterol H relative to VSMOW).

Acetylation is attractive from practical and theoretical points of view. It avoids introduction of fluorine into the analytical train, with potentially undesirable effects related to the formation and transmission of HF. It introduces only three H atoms, and the resulting ester is usually more stable against hydrolysis than are trimethylsilyl ethers which contain nine H atoms in addition to those initially present. During acetylation, no bonds to H retained in the product are made or broken, either in the derivatizing reactant (acetic anhydride) or in the substrate (a sterol or *n*-alkanol). Therefore, there is no opportunity for expression of a primary hydrogen isotope effect, with resultant, large isotopic fractionation. Secondary isotope effects associated with substrate H, if any, cannot cause fractionation because the product is formed quantitatively. Secondary isotope effects associated with acetate H, however, can lead to fractionation. The values of δD for steroidal H calculated on the assumption that acetate H is unfractionated (Table 3, column 5) are, in all cases, lower than those obtained by other techniques (columns 3 and 4). The average difference, 4.4‰ (SD = 3.4‰, $n = 5$) is at the margin of significance but is consistent with a secondary isotope effect of 6% (i.e., an effect which causes the acetate H to be depleted in D by 60‰ relative to the H in the acetic anhydride). Further work, leading to more precise estimates of this effect, is required. The present results incorporate no correction for secondary fractionation. The best available estimate of the resulting inaccuracy is 4.4 ± 4.2‰ (95% confidence interval).

3.2.2. Continuous-flow vs. conventional analyses

Sterols from Great Pond were analyzed by using both preparative capillary gas chromatography and irmGCMS. Stenol-stanol pairs could not be resolved completely under PCGC conditions. Additionally, the off-line analyses required much larger amounts of material than the on-line analyses. As a result, the best available comparisons are those presented in Table 4. For these samples, stanols comprised <25% of each stenol-stanol pair, and $28\Delta^5$ and $28\Delta^0$ are roughly three times more abundant than $29\Delta^{5,22}$ and $29\Delta^{22}$. Moreover, all of these compounds are inferred to be from similar sources, that is phytoplankton. The results are identical within analytical error, confirming the adequacy of the irmGCMS procedures and calibrations.

3.3. Terrestrial vs. Aquatic Biomarkers

Concentrations of sterol biomarkers from samples in the Great Pond watershed are summarized in Table 5. Surface sediments from Great Pond yielded a considerable variety of sterols. Among these, $d\Delta^{22}$, $d\Delta^0$, $28\Delta^5$, and $29\Delta^{5,22}$ were found only in the lacustrine sediments and appear to have no terrestrial sources. This is consistent with prior observations that these compounds are produced by phytoplankton (Volkman et al., 1998). The presence of $27\Delta^5$ and $29\Delta^5$ in suspended particulate organic matter (POM) as well as in terrestrial soils is consistent with aquatic as well as terrestrial sources for these compounds. Aquatic producers have been identified for each of these compounds (Volkman et al., 1998).

Similar sterol distributions were observed in two arctic watersheds (Avalaqiak Lake and Brother-of-Fog Lake) where terrestrial vegetation is dominated by mosses, grasses and

Table 4. Comparison of δD values measured using irmGCMS and PCGC.

Preparative capillary GC + conventional analysis[a]		irmGCMS	
Compound	δD_{VSMOW}, ‰[b]	Compound	δD_{VSMOW}, ‰
$28\Delta^5 + 28\Delta^0 + 29\Delta^{5,22} + 29\Delta^{22}$	−218 ± 6	$28\Delta^5$	−215 ± 11
$29\Delta^5 + 29\Delta^0$	−179 ± 6	$29\Delta^5$	−178 ± 6

[a] Compounds trapped from chromatographic effluent, then combusted to yield H_2O which was reduced to H_2 by use of uranium.
[b] Corrected for H added by derivatization. Reported uncertainties are 95% confidence intervals based on the number of isotopic measurements made for each sample.

sedges (Porsild and Cody, 1980). The suite of sterols found in sediments from these arctic lakes included $27\Delta^5$, $28\Delta^5$, $29\Delta^{5,22}$, and $29\Delta^5$, whereas terrestrial vegetation from these sites contained only $29\Delta^5$ and soils contained no detectable sterols. Therefore, we conclude that $28\Delta^5$ and $29\Delta^{5,22}$ can be used as aquatic biomarkers in these lakes.

Long-chain, *n*-alkyl lipids were also found in these samples. Leaves of vascular plants contain straight-chain lipids in epicuticular wax (Eglinton and Hamilton, 1967; Tulloch, 1976), and terrestrial vegetation is generally considered the major source of long ($>C_{24}$), *n*-alkyl lipids in aquatic environments (Cranwell, 1982; Meyers and Ishiwatari, 1993). Although the distribution of *n*-alkyl lipids in emergent aquatic vegetation at the margins of lakes can resemble that of terrestrial vegetation (i.e., with maximum abundances of homologues with 24 to 28 carbons, Cranwell et al., 1987; Spooner et al., 1994), the lakes in this study had little or no emergent vegetation. Thus, the primary sources of *n*-alkyl lipids are presumed to be phytoplankton and bacterial (aquatic) and higher plants (terrestrial).

3.4. Hydrogen Isotope Ratios in Biomarkers

Hydrogen isotopic compositions of lipids isolated from sediments of Great Pond are graphically summarized in Figure 1. The major feature is an enrichment of D in the *n*-alkanes and *n*-alkanols relative to sterols. Comparing lipid classes (Estep and Hoering, 1980) or individual compounds within single organisms (Sessions et al., 1999), earlier workers have observed similar enrichment. Therefore, it is likely that much or all of this isotopic difference reflects biosynthetic control of initial values of δD. Fractionation between *n*-alkyl lipids and sterols observed by Sessions et al. (1999) in living organisms was 20 to 110‰. The apparent fractionation factors observed here (20–70‰) are similar, although the sedimentary lipids in this study may include compounds of mixed terrestrial and aquatic origin.

Results of analyses of sterols and pentacyclic triterpenoids are listed in detail in Table 6. For Great Pond, two groups are evident. The first group is $27\Delta^5$, $28\Delta^{5,22}$, $28\Delta^5$, $29\Delta^{5,22}$, and dinosterol ($d\Delta^5$) with δD of −208 to −220‰. Based on the distribution of 4-desmethyl sterols in the Great Pond watershed (i.e., Table 5 and the accompanying discussion) and on the inclusion of dinosterol (a distinct algal sterol) in this group, these compounds appear to be dominated by contributions from aquatic sources. The other group includes $29\Delta^5$ and three pentacyclic triterpenols (taraxerol, α-amyrin, and one unidentified compound), with δD values of −171 to −178‰. The latter group are all components of higher plants and reflect terrestrial inputs. Based on these δD data, we infer that $29\Delta^5$ (β-sitosterol) is predominantly derived from terrestrial sources. In field and laboratory experiments, hydrogen isotopic enrichment in leafwater of 20 to 80‰ relative to soil water is commonly observed (Ziegler, 1989; Terwilliger and DeNiro, 1995). Leaf litter is observed on the bottom of Great Pond, and therefore must be a large source of terrestrial organic matter to the sediment. Enrichment of D in terrigenous triterpenols by ~30‰ relative to aquatic sterols is consistent with previous studies.

Sterols released from polar lipids by transesterification were analyzed because of evidence from marine settings which indicates that the distribution of sterols in phorbin steryl esters more closely resemble distributions in primary producers than do free sterol distributions (King and Repeta, 1994; Harradine and Maxwell, 1998). Thus, we hypothesized that sterols released by transesterification of lacustrine sedimentary lipids

Table 5. Concentrations of sterols in the Great Pond watershed.

Sample	Units	$27\Delta^5$	$28\Delta^5$	$29\Delta^{5,22}$	$29\Delta^5$	$d\Delta^{22}$	$d\Delta^0$
		Terrestrial environment					
Deciduous leaves	μg/g	0.6	0.0	0.0	202.8	0.0	0.0
Pine needles	μg/g	0.0	0.0	0.0	165.2	0.0	0.0
Soil, 0–5 cm	μg/g	7.1	0.0	0.0	38.6	0.0	0.0
Soil, 5–10 cm	μg/g	0.3	0.0	0.0	2.4	0.0	0.0
Soil, 25–35 cm	μg/g	0.0	0.0	0.0	0.3	0.0	0.0
		Aquatic environment					
POM	Relative[a]	0.1	0.5	1.0	0.7	0.0	0.0
Surface sediment	μg/g	2.5	6.7	15.8	27.6	9.8	12.0

[a] Concentration of each sterol relative to most abundant sterol.

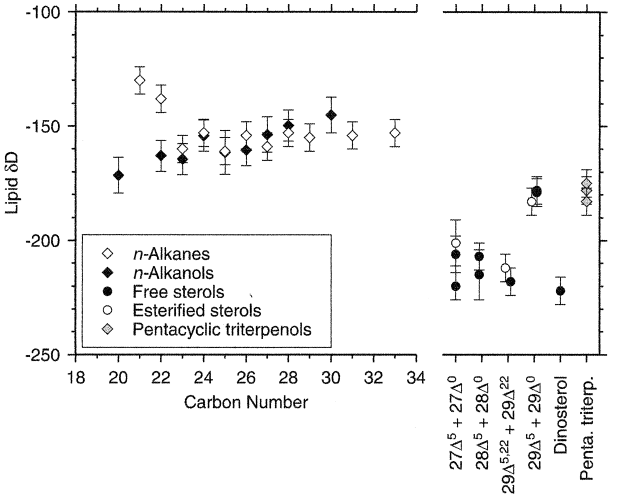

Fig. 1. Summary of lipid D/H data from Great Pond sediments. Values have been corrected for the contribution of derivative H, and are reported in ‰ relative to VSMOW. Error bars are 95% confidence limits.

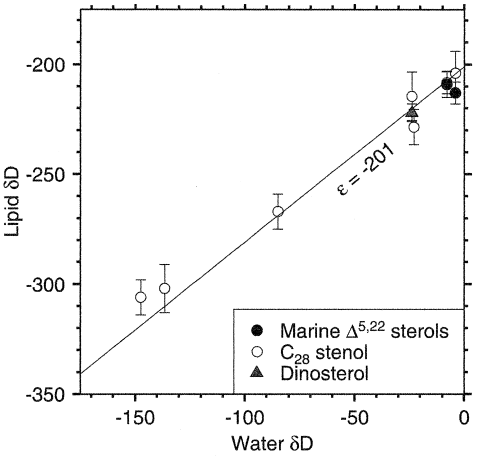

Fig. 2. Summary of D/H ratios of phytoplanktonic sterols from a suite of marine and freshwater sites. Values have been corrected for the contribution of derivative H and are reported in ‰ relative to VSMOW. Line represents constant fractionation (ε). Error bars are 95% confidence limits.

might better reflect sourcewater δD than do free sterols. However, in Great Pond, the δD of esterified sterols closely matched that of free sterols. Whereas esterified $28\Delta^5$ and $29\Delta^{5,22}$ sterols have δD values compatible with a common aquatic source, the δD of esterified $29\Delta^5$ matches that of terrestrial input (Table 6). Thus, steryl esters do not seem to be exclusively aquatic, at least in small lakes like Great Pond. We did not confirm that esterified sterols in the polar extractable lipids were in fact phorbin steryl esters: these components may have been esterified to other lipids.

The relationship of δD of sedimentary lipids to environmental water is shown in Figure 2, which is based on all aquatic products listed in Table 6. This figure assesses how reliably

Table 6. δD values of triterpenoid alcohols. Values are expressed in ‰ relative to VSMOW. Reported uncertainty estimates are 95% confidence intervals.

	Santa Monica Basin	Mud Patch	Duck Pond	Great Pond	Lac Hertel	Brother-of-Fog	Avalaqiak Lake
Water δD	−9	−10	−23 ± 2	−24 ± 2	−85 ± 4	−139 ± 2	−147 ± 2
Free sterols							
$26\Delta^{x,y}$	−209 ± 7 (5)						
$27\Delta^{x,y}$ (I)	−207 ± 6 (6)						
$27\Delta^{x,y}$ (II)	−216 ± 6 (6)						
$27\Delta^{x}$	−207 ± 11 (2)						
$27\Delta^5$	−227 ± 7 (5)	−220 ± 11 (2)	−232 ± 8 (4)	−206 ± 8 (4)	−274 ± 11 (2)	−311 ± 11 (2)	
$27\Delta^0$	−220 ± 9 (3)						
$27\Delta^5 + 27\Delta^0$				−220 ± 6[a]			
$28\Delta^{x,y}$	−218 ± 5 (9)	−208 ± 11 (2)					
$28\Delta^5$	−190 ± 9 (3)		−228 ± 8 (4)	−215 ± 11 (2)	−267 ± 11 (2)	−302 ± 11 (2)	−306 ± 8 (4)
$28\Delta^0$	−220 ± 9 (3)						−302 ± 6 (6)
$29\Delta^{x,y}$ (I–IV)	−208 ± 7 (5)	−199 ± 11 (2)					
$28\Delta^5 + 28\Delta^0 + 29\Delta^{5,22} + 29\Delta^{22}$				−218 ± 6[a]			
$29\Delta^5$	−214 ± 6 (6)	−191 ± 11 (2)	−210 ± 8 (4)	−178 ± 6 (7)	−252 ± 11 (2)	−286 ± 11 (2)	−282 ± 8 (4)
$29\Delta^0$	−224 ± 9 (3)						−276 ± 6 (6)
$29\Delta^5 + 29\Delta^0$				−179 ± 6[a]			
Dinosterol				−222 ± 6[a]			
Esterified sterols							
$27\Delta^5 + 27\Delta^0$				−201 ± 6[a]			
$28\Delta^5 + 28\Delta^0 + 29\Delta^{5,22} + 29\Delta^{22}$				−212 ± 6[a]			
$29\Delta^5 + 29\Delta^0$				−183 ± 6[a]			
Pentacyclic triterpenoids							
taraxerol				−178 ± 6[a]			
α-amyrin				−175 ± 6[a]			
unidentified pentacyclic triterpenoid				−171 ± 6[a]			

[a] Hydrogen isotopic ratio determined by preparative capillary GC/conventional dual inlet mass spectrometry.

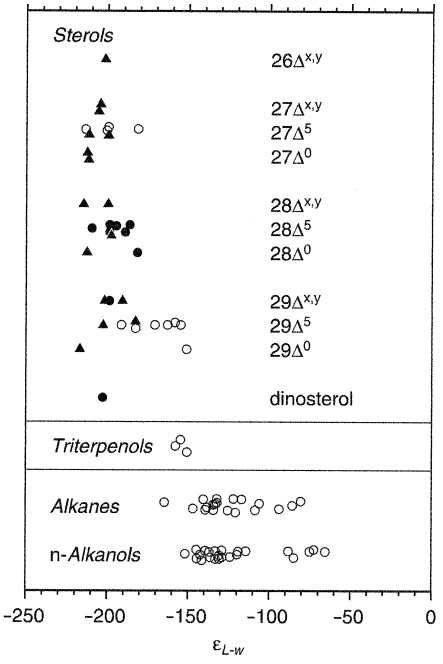

Fig. 3. Values of ε between sedimentary lipids and environmental water. $\varepsilon \equiv [(1000 + \delta D_L)/(1000 + \delta D_w)]/1000 + 1$, where L and w refer to lipids and water, respectively. Values are reported in ‰ relative to VSMOW. Triangles indicate marine sites; circles represent lacustrine sites. Filled symbols represent aquatic biomarkers, open symbols represent compounds of terrestrial or mixed origin. Before calculating ε, lipid δD values were corrected for the contribution of derivative H.

various lipids record environmental water δD in a range of environments. The lakes in this study cover a range of productivity and sedimentation rates, and occupy watersheds with greatly differing terrestrial vegetation, from forested watersheds in temperate environments (Great Pond, Duck Pond, Snow Pond) to arctic tundra near the Arctic Circle (Avalaqiak Lake, Brother-of-Fog Lake) with one site in between (Lac Hertel). Lakewater δD ranged from -23 to -147‰. Water δD values for the Mud Patch and Santa Monica Basin sites were calculated for averages of summer surface water samples (DeNiro and Epstein, 1981).

The average apparent fractionation between sterols and environmental water ($\varepsilon_{st/w}$) is -201‰ [$\varepsilon_{a/b} \equiv 1000(\alpha_{a/b} - 1)$ and $\alpha_{a/b} \equiv (\delta_a + 1000)/(\delta_b + 1000)$]. By using this fractionation factor, we predict the relationship $\delta D_{st} = \alpha_{st/w}\ (\delta D_w + 1000) - 1000$, where *st* and *w* refer to sterol and water, respectively, and $\alpha_{st/w} = 0.799$. The resulting, constant-fractionation line is shown in Figure 2. Almost all samples lie within analytical uncertainty of this line. This close correspondence indicates that environmental water D/H is the primary variable controlling D/H of algal sterols in a variety of environments. Some of the scatter around this line may reflect uncertainty in the long-term D/H of waters in the study sites, which is much larger for the arctic sites than the midlatitude lakes and marine sites.

Observed ε values for all marine and freshwater samples are shown in Figure 3. Several patterns emerge that reveal controls on the hydrogen isotopic composition of biomarkers. Multiple sterols were analyzed from two marine environments with

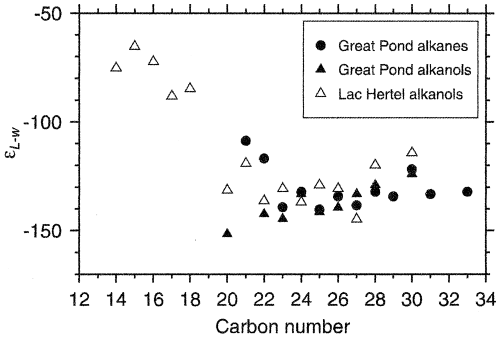

Fig. 4. Summary of D/H ratios of *n*-alkyl lipids from two lake sediments. Values for *n*-alkanols have been corrected for the contribution of derivative H, and are reported in ‰ relative to VSMOW.

limited terrestrially-derived influx (Santa Monica Basin and the Mud Patch; Fig. 3; Table 6). Both sites underlie productive surface waters, but they differ with regard to bottom-water oxygenation. The sediment-water interface at the Mud Patch is well oxygenated, whereas bottom waters in the deeper parts of the Santa Monica Basin are suboxic, rendering the sediment anoxic within several mm below the surface (Christensen et al., 1994). Thus, phytoplanktonic biomarkers deposited in the two sites differ with respect to oxygen-exposure time, a factor which appears to control burial efficiency (Hedges et al., 1999). Whether because of differences in productivity or water column and sediment anoxia, sterols are preserved in greater diversity and higher abundance in Santa Monica Basin than in the Mud Patch. Based on ^{13}C and ^{14}C evidence from the same Santa Monica Basin sediment sample, Pearson (1999) concluded that these compounds are derived almost entirely from phytoplanktonic sources and that terrestrial sources are negligible. The δD data support this interpretation. In the Mud Patch, similar values for δD indicate that sterols in this environment are mostly marine. The identical δD values for sterols of planktonic origin imply that different primary producers all impart similar fractionation factors. Because of this, any phytoplanktonic sterol can potentially be used as a tracer for sourcewater δD, allowing multiple proxy measurements for samples that contain multiple phytoplanktonic sterols.

Apparent fractionation factors for *n*-alkyl lipids are more variable, ranging from -65 to -165‰ (Figs. 3 and 4). All *n*-alkyl lipids are more enriched in D than co-occurring sterols. Two factors may contribute to these differences. Recent D/H measurements of individual compounds have provided new insights into the primary controls on lipid H isotopic composition (Sessions et al., 1999). In particular, they have shown that different classes of lipids exhibit fractionation factors that differ significantly. Key factors appear to include not only structure (acetyl vs. isoprene based carbon skeleton) but also site of synthesis (chloroplast vs. cytosol).

Differences between sedimentary *n*-alkyl lipids and sterols may also reflect different sources for these compounds. In surficial sediments, δD values of *n*-alkyl lipids are more tightly constrained for longer chain lengths ($>C_{22}$) at certain sites (Fig. 4, likely reflecting a similar source for these compounds (i.e., higher-plant leaf waxes). Isotopic compositions of *n*-alkyl lipids with shorter chain lengths (C_{14} to C_{22}) are more variable. At Lac Hertel, short-chain *n*-alkanols (C_{14} to C_{18}) are enriched

in deuterium by 40 to 60‰ relative to all other *n*-alkyl lipids, indicating a different source for these compounds. This finding is surprising because phytoplanktonic and aquatic bacterial sources of short-chain *n*-alkyl lipids are generally considered to predominate over terrestrial inputs (Meyers, 1997), with subsequent D-depletion relative to terrestrially sourced lipids. Because short-chain *n*-alkyl lipids are important components of living biomass, surficial sediments may contain large proportions of actively cycling biochemicals. *n*-Alkyl lipids extracted from living organisms suggests there may be multiple pools of H within living cells with distinct isotopic composition (Sessions et al., 1999), which may not contribute equally to sedimentary lipid pools. The apparent ε values we observed for C_{14}-C_{18} *n*-alkanols (-65 to -85‰) are less negative than previously observed for any lipids in cultures or field specimens (about –150‰, Sessions et al., 1999). Although a full understanding of the controls on the H isotopic composition of sedimentary *n*-alkyl lipids will require further investigation, our data indicate that different compounds are associated with different apparent fractionation effects, and factors other than environmental water affects the H isotopic composition of some biomarkers. Although the H isotopic composition of these compounds may provide valuable information regarding the metabolism of the source organism, they must be avoided in studies attempting to reconstruct environmental water δD.

The isotopic compositions of phytoplanktonic sterols in marine and freshwater systems are related to source-water δD. For example, the mean ε for $28\Delta^5$ is -194 ± 10‰ ($n = 6$). When all marine and freshwater aquatic sterols are pooled, the mean ε is -201 ± 12‰ ($n = 22$). Thus, hydrogen-isotopic compositions of aquatic sterols allow estimation of D/H ratios of environmental waters to within 10 to 12‰ (Fig. 3). The consistent ε values observed here in sedimentary sterols contrast with more variable fractionations observed in living plants, bacteria and algae (Sessions et al., 1999). Either the sediments are dominated by less variable products that are preserved preferentially or the averaging inherent in accumulation of sediments neutralizes short-term variations that can be observed in living organisms.

In freshwater systems, $29\Delta^5$ is usually enriched by 15 to 40‰ relative to the aquatic sterols (Table 6), probably reflecting the influence of terrestrial sources. The precision of our measurements of sterol δD values is limited to ± 10‰ because of the presence of closely eluting compounds. We expect that enhanced chromatographic sterol separation and improvements in the technical design of the GC and pyrolysis reactor will result in more precise measurements in the future.

The use of specific biomarkers for reconstructing environmental D/H complements and expands existing paleoenvironmental approaches. To reconstruct isotopic compositions of paleo-lakewaters, it is necessary to isolate from sediments a component which is unaffected by nonlacustrine processes such as evaporation from emergent leaves or from ground surfaces. Carbonate minerals in aquatic fossils or marly encrustations of aquatic plants are recorders of water δ^{18}O (Kelts and Talbot, 1990). In special cases, organically bound O can be used (Sauer et al., in press). In particular, sedimentary cellulose from aquatic plants has been analyzed as a proxy for lakewater isotopic composition (Edwards and McAndrews, 1989; Wolfe et al., 1996). Based on surface-sediment calibrations and on comparisons of downcore records with other proxies for isotopic compositions of meteoric waters, several studies have concluded that cellulose accurately records the oxygen isotopic composition of lakewater in a variety of lacustrine environments (e.g., Edwards and McAndrews, 1989; Wolfe and Edwards, 1997).

Krishnamurthy et al. (1995) have interpreted a isotopic record of bulk hydrogen in lacustrine kerogen in terms of variations in the isotopic composition of lake water. However, organic fractions such as kerogen and cellulose can have significant allochthonous components, and terrestrial organic matter is often isotopically enriched in deuterium relative to aquatic organic matter because of two processes. Because elemental ratios of carbon to nitrogen (C/N) are lower for aquatic biomass (C/N = 6–10) than for terrestrial biomass and soils (>20, Meyers and Ishiwatari, 1993), low C/N ratios in sedimentary organic matter are often cited as evidence of autochthonous origin. However, C/N ratios can be affected by selective degradation and microbial reworking, precluding any straightforward interpretation of sedimentary C/N ratios. Studies in both marine and lacustrine environments have used ^{14}C and lignin phenol concentrations as evidence of terrestrial inputs, and have concluded that C/N ratios lower than 10 can occur even where organic carbon is largely terrigenous (Goñi et al., 1998; Sauer et al., in press).

It is important to exclude terrigenous organic matter because evaporation from leaves causes deuterium enrichment of the leafwater that is recorded by photosynthetic products (e.g., Terwilliger and DeNiro, 1995). Also, where snow comprises a major component of annual precipitation, snowmelt can be delivered quickly to lakes and streams, effectively bypassing the terrestrial ecosystem. Summer rains are usually enriched in D and ^{18}O relative to winter snow, and terrestrial vegetation that grows utilizing summer precipitation is likewise enriched relative to aquatic vegetation that is derived from water reflecting a longer-term average isotopic composition of meteoric water. Because terrestrial vegetation is often significantly enriched in D and ^{18}O relative to lacustrine organic matter, it is not possible to extract meaningful paleoclimatic information from bulk measurements of sediments with large and variable inputs of terrigenous cellulose. For such sites as these, it is necessary to derive more selective means of isolating aquatic organic matter.

Studies of biomarker D/H for environmental problems make use of the wide range of D/H observed in natural waters. δD values for total annual precipitation range from ~0‰ in some tropical regions to less than –150‰ in high mountains and at high latitudes (Dansgaard, 1964; Rozanski et al., 1993). Differences in hydrogen isotopic composition recorded in insect wings and bird feathers have provided insights to migration patterns across D/H gradients (Hobson and Wassenaar, 1997; Hobson et al., 1999). In these studies, Hobson et al., used underivatized animal tissues that included potentially exchangeable hydrogen and found convincing correlations to D/H of environmental water. Applying single-compound approaches to these problems might improve resolution. Lakewater D/H changes seasonally because of differences evaporation, precipitation, and snowmelt inputs, which can affect lakewater D/H by >40‰ (Gonfiantini, 1986; Gibson et al., 1994; Sauer, 1997). Because the environmental gradients are

large (often >100‰) and exceed our analytical uncertainties, the techniques described in this paper could be applied to similar problems.

4. CONCLUSIONS

Hydrogen isotope ratios in lipid biomarkers record the isotopic composition of environmental water. Certain sterols in freshwater systems can serve specifically as aquatic biomarkers, and can be used to reconstruct lakewater δD to within ±10‰. The observation of indistinguishable, if not identical, fractionation factors between water and different aquatic sterols extracted from both freshwater and marine sediments suggests that sterols derived from phytoplanktonic sources can be used interchangeably to reconstruct sourcewater δD in different environments. The measurement of unambiguously aquatic biomarkers avoids the inherent uncertainty in the use of sedimentary organic substrates or fractions that are derived from both terrestrial and aquatic sources.

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